

NASA-CR-194402

1N-51-CR  
OCIT  
186117  
40P

**FINAL REPORT**

NAWG-2739

October 1, 1991 - September 30, 1993

---

**CELLULAR RESPONSES TO LOW-GRAVITY: PILOT STUDIES ON  
SUBORBITAL ROCKETS AND ORBITING SPACECRAFT"**

---

(NASA-CR-194402) CELLULAR  
RESPONSES TO LOW-GRAVITY: PILOT  
STUDIES ON SUBORBITAL ROCKETS AND  
ORBITING SPACECRAFT Final Report, 1  
Oct. 1991 - 30 Sep. 1993 (Alabama  
Univ.) 40 p

N94-14494

Unclass

G3/51 0186117

Submitted by

Marian L. Lewis, Ph.D.  
Consortium for Materials Development in Space  
University of Alabama in Huntsville  
Huntsville, AL 35899

Phone (205) 895-6391  
FAX (205) 895-6376

## **FINAL REPORT**

**FOR PERIOD OCTOBER 1, 1991 - OCTOBER 1, 1993  
91-422 (NAWG-2739)**

### **"CELLULAR RESPONSES TO LOW-GRAVITY: PILOT STUDIES ON SUBORBITAL ROCKETS AND ORBITING SPACECRAFT"**

Submitted by  
Marian L. Lewis, Ph.D.  
September 7, 1993

#### **SUMMARY**

The allocated funding supported, in part, experiments conducted on two Consort sounding rockets and five Shuttle flights. The primary parameters investigated were signal transduction in response to various mediators, cellular differentiation and metabolism in microgravity and effect of microgravity on cytoskeletal morphology. Achievements include: demonstration of effect of spaceflight on the actin cytoskeleton in mouse osteoblasts (collaborator, M. Hughes-Fulford) and frog cells (collaborator, R. Gruener); confirmation that the T cell receptor-mediated signal transduction pathway in T lymphocytes is not affected by low-gravity compared to non-TCR-mediated stimulation (Con-A) which classically does not promote proliferative response; indication that microgravity may allow separation of proliferative signaling and secretory function in lymphocytes (collaborator, M.A. Principato); demonstration that T lymphocytes and bone cells utilized less glucose indicating a shift in metabolism and confirming Spacelab results with WI-38 cells which used significantly less glucose, during spaceflight; confirmation that activation of human splenic B cells with a number of different mediators is not affected during spaceflight (collaborator, G. Neil); demonstration of increased prostaglandin synthesis during reduced bone cell growth suggesting an effect of microgravity on prostaglandin-induced mitogenesis (collaborator, M. Hughes-Fulford).

The funding contributed significantly to the database described above and resulted in submission of six collaborative abstracts (attached) in 1993 (five to the ASGSB Annual Meeting and one to the ASCB Annual Meeting). Two abstracts were presented at the 1992 ASGSB Annual Meeting in Tucson. In addition, several peer reviewed papers are being generated and data will be included as background in preparation of future proposals, which hopefully will allow us to continue this type of extremely productive collaborative research.

(Note: Achievements resulting from collaboration with selected investigators is described in the text):

## **INTRODUCTION**

This final report describes the activities conducted during the one year plus one year extension granted by NASA Headquarters Life Sciences for the purpose of acquiring additional data from flight experiments flown in 1991, 1992 and 1993. A summary of all flight results and cell response data obtained from this \$10,000 grant is included. This funding supported significant cell biology research, allowed me to coordinate specific collaborations to achieve research goals, and maximize capability to conduct basic research on space flights already available through my position in the Consortium for Materials Development in Space (CMDS) at the University of Alabama in Huntsville (UAH). Through my collaborations with selected investigators and their "in kind" support, this funding was greatly expanded to provide very significant low-gravity cellular response data. The primary advantage of this enhanced co-operative activity is frequent access to space, collaboration with investigators in related research areas leading to rapid acquisition of data, sufficient flight opportunities to allow confirmation of data and adequate replication of experimental parameters.

The obvious wealth of information, gained in part from this two-year funding, illustrates the extreme value of co-operative interaction between NASA's Life Science Program and the NASA Centers for Commercial Development of Space (CCDS). The CCDS program provided repeated access to space for multiple experiments, multiple replicates, and multiple collaborative investigations. These factors led to a rapid database expansion for a variety of cell types by maximizing low-g access and providing relatively low-cost, frequent, multiple-user opportunities. The benefit to the CMDS is expansion of the cell biology database on low-gravity response to identify target products and processes which can lead to commercial space bioprocessing and enhanced quality of life on Earth. The benefit to NASA's life sciences goals is expansion of the understanding of fundamental cellular processes in altered gravity environments.

## **PROJECT REPORT**

**Research Objectives:** The primary objective of this research was to advance knowledge of molecular control mechanisms during cellular differentiation and to gain understanding of mechanisms of cellular responses in low-g. The specific objectives were to evaluate cytoskeletal structure in cells exposed to low-g compared to static culture ground-controls and to gain information on early signal transduction events and gene expression. These parameters were selected for investigation because of the reports in the

literature indicating that some low-gravity effects occur within 10 minutes after stimulation of cells. These responses would thus be detectable for cells flown on sounding rockets as well as the Shuttle.

The specific objectives of this pilot study were to maximize rocket and orbital spaceflight opportunities to gain information on mechanisms involved in altered response of human lymphocytes in low-g and to determine if similar mechanisms are also altered in other cell types. Additional objectives were to evaluate the available hardware for this research and to develop procedures to evaluate cells from more detailed experiments planned for future flights.

**Hypotheses tested:** All terrestrial life and Earth-based processes have evolved under unit gravity conditions and are therefore designed to make optimum use of gravity. For instance, the countless single events which occur in a living cell during growth, differentiation and gene expression have all evolved to take advantage of the unit gravity environment on Earth. In theory, pathways of development would have been very different if organisms had evolved in the absence of gravity. This is validated by increasing numbers of reports, as low-gravity research becomes more frequent and sophisticated, that gene expression, metabolism and cellular functions are affected during spaceflight. We hypothesized that alteration in gravity environment would produce detectable changes in the cytoskeleton and signal transduction pathways resulting in altered response of cells to growth mediators during spaceflight.

### **Collaborations**

This research has been achieved through collaboration with outstanding investigators who have contributed some reagents and analyses on their equipment in return for opportunities to fly their cells collaboratively with me in order to achieve our common objectives. Obviously the \$10,000 funding could not possibly cover all of the work described in this final report; however, the funding made it possible for me to purchase some reagents and materials in support of the cell investigations described and to travel to the ASGSB annual meeting where collaborations were established and plans for research were discussed. The funding also provided some salary support allowing me to maximize already available access to space through the UAH CMDS sponsored by the NASA Office of Advanced Concepts and Technologies (OACT), to gain the utmost science benefit from my spaceflight opportunities. This expanded report is presented to illustrate the volume and good quality of research that can be achieved from combined support of NASA's space commercialization and basic research activities.

### Primary collaborators

Millie Hughes-Fulford, Ph.D., Astronaut and Chief, Laboratory for Cell Growth and Differentiation, Department of Veterans Affairs Medical Center, Univ. CA.San Francisco. (3T3 Mouse bone cell differentiation and growth).

B. DeSales Lawless, Ph. D., Dept. of Cellular Immunology, Rockefeller Univ. New York (Cellular immunology and flow cytometry).

David W. Sammons, Ph.D., Department of Microbiology, Univ. Arizona, Tucson, AZ (B cell immunology and fixative investigations).

Garry Neil, M.D. Oakdale Research Park, Univ. Iowa, Coralville, IA (Splenic B cell differentiation and immunology).

Raphael Gruener, Ph.D., College of Medicine, Department of Physiology Univ. AZ Med School, Tucson, AZ (Xenopus cytoskeleton morphology and metabolism).

Augusto Ochoa, M.D., Dept, Clinical Immunology, Frederick Cancer Treatment and Dev. Cntr. Frederick MD. (Human T lymphocyte immunology).

Mary Ann Principato, Ph.D., Immunology Branch, Food and Drug Administration, Laurel MD. (Mouse T lymphocyte immunology).

## **SUMMARY OF ACCOMPLISHMENT OF INDIVIDUAL PROJECT OBJECTIVES ORIGINALLY PROPOSED FOR THIS PILOT STUDY NASA FUNDING**

**Cytoskeleton Evaluations.** Gross morphology of the cytoskeleton was evaluated by immunofluorescent microscopy to determine if the elements are disorganized, if they extend to the cell membrane, if regulatory proteins (i.e. protein kinase C) are associated with cytoskeletal filaments and if stress fibers are visible. This objective was originally intended for human lymphocytes. Because the equipment needed to evaluate the lymphocytes was located at Birmingham and the collaborator accepted a position elsewhere, this part of the intended study was shifted to collaborators working with different cells, i.e. mouse osteoblasts (Millie Hughes-Fulford) and *Xenopus* cells (Raphael Gruener). The objectives were met in that we showed that the gross morphology of the actin cytoskeleton is significantly altered in low-g in both mouse and frog cells. This objective was accomplished through cooperation with Drs. Hughes-Fulford and Raphael Gruener. We did not evaluate translocation of protein kinase C along the cytoskeletal elements. This remains to be done.

**Antigen-antibody binding in low-g.** The demonstration of binding of antibodies to cell membranes is significant because it allows us to identify cell surface markers and evaluate membrane binding by ligands in low-g. Mixing cells with antibody specific to a particular cell surface marker to determine if binding is comparable to that in 1-g (i.e. if cell surface receptors are altered by low-g) was achieved on STS-43. (This was attempted on Consort-4, but the hardware anomaly prevented evaluation of this parameter). On STS-43 we demonstrated comparable flight and ground binding of stem cell antigen (SCA-1) antibody and Thy-1 antibody to mouse bone marrow stem cells and also demonstrated that our hardware (the MDA Minilab) is adequate for this type of cell test.

**Mechanisms of T lymphocyte response to mitogen activation in microgravity.** A primary objective of this research was to determine why cells do not respond well to Con A stimulation during spaceflight. Our experiments with human T lymphocytes confirmed low Con A activation but significant secretion of cytokines. We showed that activators of T cells which are mediated through the T cell receptor (TCR) were not altered in low-g, though Con A response is altered. Thus, we have confirmed by inference that the reduced Con-A activation is a result of early membrane associated reactions, probably involving phospholipase C as suggested by work of Schaffar *et al* using phorbol esters. The following abstract, submitted to the annual meeting of the American Society for Cell Biology (ASCB) summarizes our lymphocyte investigations.

EFFECTS OF MICROGRAVITY ON LEUKOCYTE GROWTH CONTROL AND FUNCTION. ((M.L. Lewis<sup>1</sup>, M.A. Principato<sup>2</sup>, B.D. Lawless<sup>3</sup>, D.R. Morrison<sup>4</sup>, W.C. Kapp<sup>5</sup>, S.L. Strobl<sup>5</sup> and A.C. Ochoa<sup>5</sup>)) Department of Biology, University of Alabama, Huntsville, AL 35899<sup>1</sup>, Immunology Branch, Food and Drug Administration, Laurel, MD 20708<sup>2</sup>, Department of Cellular Immunology, Rockefeller University, NY, NY 10021<sup>3</sup>, NASA Johnson Space Center, Houston, TX 77058<sup>4</sup>, and Program Resources Inc./DynCorp, Frederick MD 21702<sup>5</sup>.

Spaceflight results in significant reduction in mitogenic activation of human T lymphocytes. Causal mechanisms are not understood though cell contact and macrophage function are implicated. We evaluated microgravity effect on lymphocyte responsiveness, cytokine secretion and signaling through the T cell receptor (TCR) complex. Human or murine T lymphocytes were exposed to: non-TCR binding Con A, an efficient activator of resting T cells in unit gravity but not in microgravity; anti-CD3 plus IL2; and superantigen Staphylococcal Enterotoxin B (SEB) in the presence of Class II presenting feeder layers in unit-gravity and on Space Shuttle flights STS-50, 52, 54, and 56. Cells were exposed to mediators for two to 94 hours followed by fixation of flown cells in microgravity. The human peripheral blood mononuclear cells (hPBMC) exposed to Con-A remained primarily in G1 during spaceflight while ground controls progressed through the replicative cycle. Glucose utilization, significantly lowered in spaceflight, confirmed reduced metabolic activity of Con-A treated cells. However, stimulation of normal hPBMC in flight with anti-CD3+IL2 resulted in activation and appreciable cytokine production (gamma-IFN, GM-CSF, IL-1beta, and IL-6) confirming previous flight results indicating significant changes in cytokine secretion. Stimulation of purified murine splenic T cells through direct engagement of the T cell receptor with the SEB superantigen or via the TCR-associated CD3 molecule using anti-CD3+IL2 was also achieved in microgravity. In both instances, the murine T cell populations transitioned into DNA synthesis (S phase) and mitotic division (G2+M), indicative of cellular proliferation. We conclude that T cell activation via the TCR-mediated pathway is unaffected by microgravity while non-TCR-mediated activation, confirmed by poor proliferative response to Con A, does not promote cell division in microgravity. Our results suggest that microgravity may permit separation of proliferative signaling and T cell responsiveness and thus provide an unparalleled opportunity to investigate basic cellular mechanisms controlling growth and function.

**Expression of c-myc and c-fos.** This evaluation is based on the report of Reed et al. (1986) that lectin-stimulated mitogenesis of normal human lymphocytes results in sequential expression of protooncogenes and that after stimulation of peripheral blood mononuclear cells with phytohemagglutinin (PHA), a marked increase (within 10 minutes) occurred in levels of mRNA for c-fos and c-myc. Also, a decrease in expression of the fos proto-oncogene in simulated low-g has been reported (Rijken et al.). Evaluation of early gene expression is feasible because the increase in mRNA occurs within 10 minutes, a time easily achieved on rockets. This objective was only partially met. We had difficulty working out the details of the Boehringer non-radioactive technique and found it to be generally unworkable in our laboratory. We are shifting our emphasis to PCR and to *in situ* detection using a radiolabeling technique. We plan to continue development of the technique for use with cells from our next three Shuttle flights and Consort rocket launches.

**Phosphatidylinositol Hydrolysis.** An early event in lymphocyte activation after receptor binding is breakdown of phosphatidylinositol-1,4,5-bisphosphate (PIP<sub>2</sub>) to inositol-1,4,5-trisphosphate (IP<sub>3</sub>) and 1,2-diacylglycerol (DAG). Modifications of this assay are in test since timed steps in the assay do not correspond exactly with the possible reagent exposure times in low-g. This technique requires short incubation with specific reagents which we could not accomplish on the Shuttle and by the time the rockets were recovered and samples returned to us, the samples were not testable. We are looking for a more workable technique. Of course, this would be achievable on Space Station where a technician could perform the required activities in real time.

## DESCRIPTION OF HARDWARE

### MATERIALS DISPERSION APPARATUS (MDA) HARDWARE

*Hardware Provider, John M. Cassanto, Instrumentation Technology Inc., Exton, PA*

The Materials Dispersion Apparatus (MDA) Minilab, (Figure 1) was commercially developed by Instrumentation Technology Associates, Inc. (ITA). The MDA Minilabs are brick-sized units consisting of two sliding blocks with sample wells cut into opposite faces. The blocks, misaligned during launch, slide to bring fluids in the wells of top and bottom blocks into contact during the low-gravity period. For the Type-3 wells, the blocks slide again before re-entry to bring the samples into contact with wells containing a fixative. Volumes in wells vary from 125 to 500 microliters depending on the diameter and depth of the wells required for specific investigations. A block may have as many as 140 well sets and thus adequate replication of test parameters and experimental variables control are easily provided.

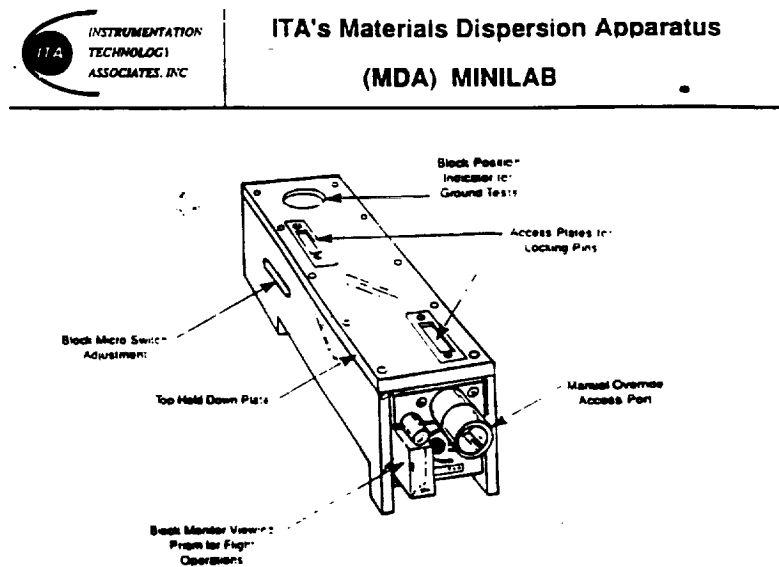


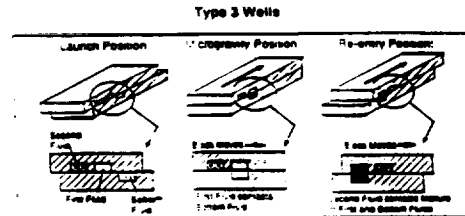
Figure 1. The ITA MDA Minilab.

Well configurations (Figure 2) allow mixing of two (Type-2) or three (Type-3) fluids in microgravity. Type 1- wells do not allow mixing of fluids and remain in the same position throughout the mission.



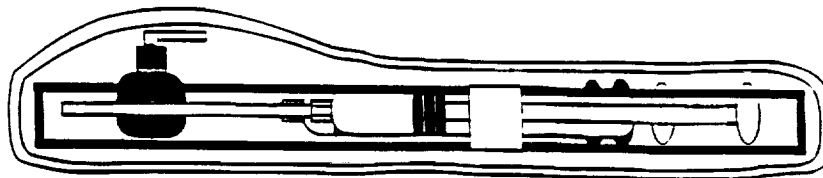
The diagram illustrates three types of welds: Lap Joint, T-Joint, and Re-entry Joint. Each diagram shows a cross-section of the joint and a corresponding detail view of the weld area.

- Lap Joint:** Shows two plates overlapping. The detail view shows a weld joint with labels: "Lap Joint", "Weld 1", "Weld 2", and "Weld 3".
- T-Joint:** Shows a plate (Plate 1) attached to the side of another plate (Plate 2). The detail view shows a weld joint with labels: "T-Joint", "Weld 1", "Weld 2", and "Weld 3".
- Re-entry Joint:** Shows a plate (Plate 1) passing through another plate (Plate 2). The detail view shows a weld joint with labels: "Re-entry Joint", "Weld 1", "Weld 2", and "Weld 3".



Several science-driven modifications to the MDA's have resulted from my requirement to fly live cells. These modifications include fabrication of blocks using clear material to increase visibility during loading and unloading wells, triple containment to allow flight of NASA-classified catastrophic fluids (chemical fixatives), strip heaters and a precision thermostat to maintain 37°C, and grooves cut into the wells to accommodate small coverslips for anchorage dependent cells. With these adaptations, the area of cell biology research in the MDA's is now possible. Another significant CMDS contribution to the refinement of hardware design to accommodate fluids/particles mixing, was the placement of magnetic discs under bottom wells and addition of magnetized particles with the cells, or other materials, in top wells.

BPM's (Figures 3, 4, 5) are included in the materials dispersion sub-category. The BPM's are simple, manually operated devices. They consist of four syringes interconnected by tubing to a



8

4-way valve. One syringe contains cells, another contains a growth factor/mediator, and two syringes contain fixative. The valve, turned by the astronaut to pre-selected positions, allows injection of fluids from one syringe into another to mix materials at specified times during the flight. The BPM's provide a larger volume (6 mls) of cells or other fluids but allow for only two samples per BPM rather than the typical MDA which provides up to 120 samples of volumes of 0.125 to 0.5 ml. BPM's are triply contained in flight-certified sealed bags to satisfy NASA safety requirements. Figure 5 shows BPM's on CMIX-1 operated by Lacy Veach.

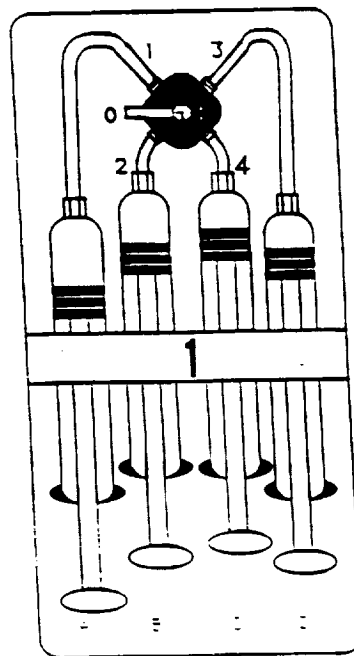


Figure 4. Bioprocessing Module (BPM) with 4-way valve.

ORIGINAL PAGE IS  
OF POOR QUALITY



Figure 5. Astronaut, Charles Lacy Veach operating BPM's on STS-52.

### Other Hardware

As a courtesy and exchange between NASA's CCDSs, I have the opportunity to fly experiments in the Fluids Processing Apparatus (FPA) (Figure 6) of BioServe. To date, this has included experiments on STS-50 and STS-54. The FPA's consist of a glass tube with two bypasses formed along opposite sides to allow fluids to mix between three chambers which are separated by septa. Tubes are placed in lexan cylinders for triple containment. Experiments are activated or fixed by pushing a plunger on a manifold.

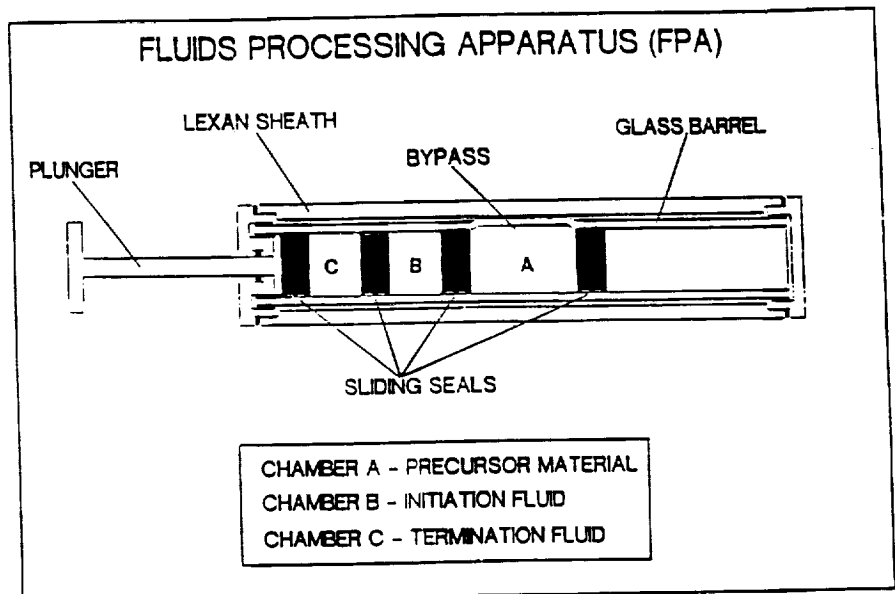


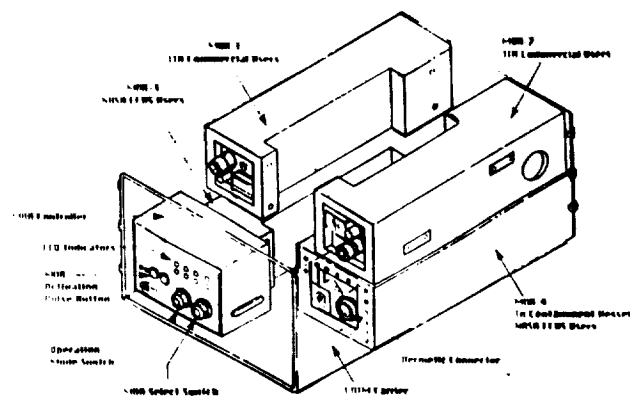
Figure 6. The BioServe CCDS Fluids Processing Apparatus (FPA).

### The Commercial Refrigerator Incubator Module (CRIM)

CRIM's are made available to the CCDS's through NASA's Office of Advanced Concepts and Technology. This controlled temperature refrigerator/incubator module fits into a Shuttle middeck locker and maintains an approximate set temperature for pre-launch and on-orbit operations. A CRIM carrier, made by ITA, fits inside the CRIM and provides attachment points for the MDA's. The BPM's are stowed in spaces between the MDA's for launch and landing and are de-stowed for on orbit operations. Figure 7 shows a diagram of the CRIM and Figure 8 shows the CRIM carrier fabricated by ITA with four MDA's, the controller and BPMs stowed as flown on CMIX-1 and CMIX-2.

Exploded view diagram of a radio receiver. The diagram shows the front panel, chassis, and speaker. The front panel is labeled "FRONT PANEL" and features a speaker grille, a tuning dial, and various control knobs and switches. The chassis is labeled "CHASSIS" and shows the internal components, including the tuning circuit, power supply, and speaker. The speaker is labeled "SPEAKER" and is shown as a separate component. The diagram is a technical drawing with dashed lines indicating the assembly path.

Page 2-1     MKT Travel



**Figure 8. CRIM with CMIX Payload.**

**STS-43 (August, 1991):** This flight in August 1991 validated the use of ITA's MDA minilabs for cell biology experiments and provided the preliminary information for the FY 1992 experiments. The STS-43 experiments were flown on BIMDA-2 (BioServe sponsored flight of ITA's MDA minilabs) as a joint BioServe/University of Colorado CCDS - ITA payload. On this flight, in addition to ITA experiments, the CMDS flew mouse bone marrow and human lymphocytes which were evaluated after flight for immunologically detectable markers. Results indicated that cells maintained their antigenic identity. The flow cytometry profiles of mouse bone marrow cells fixed after one day in low-g and evaluated to identify the cell type by surface markers show comparable populations of stem cell antigen (SCA-1) and the immune cell marker (Thy-1) positive cells for both Earth-based and spaceflight cells. This significant result validated the MDA's for experiments with live cells. Summarized results of the various experiments flown on the BIMDA Payload are shown in Table 1.

## STS-43 MDA Experiment Results

FIELD OF STUDY/APPLICATION	EXPERIMENT	FLIGHT RESULTS
Biomedical/Aging	Brine Shrimp Development	Unsuccessful. Dry shrimp never contacted water. 1st time tried in MDA.
Biomedical/Aging	Wasp Development	1 wasp hatched on orbit and was alive out of 50 wasp pupae.
Biomedical/Biophysics	Bone Matrix Formation	Decalcified bone digested during flight.
Biomedical/Biophysics	Bone Decalcification	Reduced rates of in vitro bone mineral removal.
Biomedical/Biophysics	Collagen Assembly on Bone Matrix	Collagen polymerization occurred on bone matrix.
Cell Biology	Macrophage Activation	Increased lymphokine production.
Cell Biology/Fixation calibration data	Cell Fixation	Fixative worked to preserve certain structures of cells in space.
Cell Biology/Plant Studies	Mustard Spinach Seed Germination	Sprouts were formed in some samples.
Cell Biology/Test of magnetic mixing concept	Cell Mixing	Magnetic mixing concept was validated. With magnetic mixing, 77.94% of cells mixed - without, only 20.38% of cells mixed.
Fluid Sciences/Biomaterial	Yeast Diffusion	Diffusion successful
Fluid Sciences/Calibration data	Particle Diffusion	Diffusion successful
Fluid Sciences/Calibration data	Trypan Blue Diffusion	Diffusion successful
Fluid Sciences/Calibration data	Diffusivity of Cells	Determined diffusion coefficients
Fluid Sciences/Diffusion	Aqueous Copper Sulfate Diffusion	Diffusion successful
Microbiology/CELSS*	Bacteria & Algae Metabolism	Not available
Molecular Biology/Artificial Implants	Collagen Assembly	Successful collagen polymerization
Molecular Biology/Pharmaceutical	Liposome Formation	Liposomes formed larger than ground controls
Molecular Biology/Pharmaceutical	Virus Assembly	Not available
Molecular Biology/Pharmaceutical	E. Coli Development	E. Coli multiplied faster than controls
Molecular Biology/Pharmaceutical	Clot Lysis	Successful lysis of fibrin clots by urokinase
Plant Biology/CELSS*	Alfalfa & Clover Seed Germination	Some sprouts formed, but overall much less sprouting than on STS-37.
Protein Crystal Growth	Bacterial Aldolase Crystal Growth	No crystals formed
	Double Diffusion Crystal Growth	Some crystals formed
	Lysozyme Crystal Growth	Large, diffraction quality crystals formed
	Osmotic Dewatering Crystal Growth	Osmotic dewatering concept validated. High quality crystals formed.
	Rabbit Aldolase Crystal Growth	Good results. 2mm pyramid-shaped crystals formed. Unlike form on earth.
Protein Crystal Growth/ Breast Cancer Research	Urokinase Crystal Growth	Small fragile crystals formed in 8 days. Too small for x-ray diffraction. On ground, it takes ~6-9 months to form same crystals.

Notes: \* CELSS = Controlled Ecological Life Support Systems (for Space Station, Lunar Base, etc...)

1. All MDA's performed successfully, no leakage or cross contamination was seen.
2. 21 out of 22 experiments were successful.

ORIGINAL PAGE IS  
OF POOR QUALITY

Table 1. STS-43 Experimental Results.

**STS-50 BioServe CCDS (June 1992):** (Flown as a CCDS to CCDS courtesy using BioServe Fluids Processing Apparatus {FPA} hardware). Human lymphocytes were flown in collaboration with Dr. Augusto Ochoa of Program Resources, Inc. at the Frederick Cancer Center in Frederick, Maryland. The cells were loaded into the BioServe Fluid Processing Apparatus (FPA) tubes (Figure 6), mixed with a growth mediator for 4 days in microgravity and then mixed with fixative. Results indicated that the cells remained sufficiently viable but did not grow when exposed to the mediator at the low temperature (25°C) on this mission. A subsequent test was conducted in January 1993 at 37°C on STS-54.

**STS-54 BioServe CCDS (January 1993):** This experiment was a repeat of the STS-50 investigation with the exception that the FPA's were maintained at 37°C, this temperature permitted lymphocyte proliferation. Identification of cell surface markers for activated cells indicated positive human cell activation by OKT-3 monoclonal antibody in the presence of exogenous IL-2 in space and ground controls. Flown cells appeared to be activated less than ground controls but secreted significant levels of specific cytokines into the medium. These results are consistent with other

data indicating retarded growth of cells in space but inconsistent with data from cells stimulated with another type of mediator (Con A) which produced higher levels of some cytokines (Interferon gamma) in microgravity compared to ground controls (Chapes, S.K., D.R. Morrison, J.A. Guikema, M.L. Lewis, and B. S. Spooner. "Cytokine production by immune cells in space". J. Leukocyte Biol. 52:104-110 (1992)). The difference in the activators used may accounts for the difference in cytokine secretion (See abstract on Page 5).

**Consort-5 Sounding Rocket (September 1992):** Live cell tests flown on Consort 5 indicated that the MDA's adapted to accommodate glass coverslips and maintain 37°C temperature performed flawlessly. The cells, including mouse bone, Xenopus (frog) muscle/nerve co-culture, and CV-1 (monkey kidney cell line), remained attached to the coverslips for the total mission profile. This was an extremely significant evaluation since the MDA's were proven to have a new capability to accommodate attachment-dependent cells to be flown on subsequent Shuttle missions.

**Table 2. CMDS EXPERIMENTS FLOWN IN THE MDA MINILABS ON CONSORT 5**

<b>SAMPLE</b>	<b>INVESTIGATOR/COLLABORATOR/LOCATION</b>	<b>RESULT</b>
Primary human lymphocytes	M./ UAH	Remained viable, MDA validated
Monkey kidney cell line	M. Lewis/ L. Binder/ UAB	No cytoskeletal data obtained
Human B lymphocytes	M. Lewis/ D. Sammons/ U. Arizona	Fixative tests feasibility
Mouse osteoblasts on cover slips	M. Lewis/ M. Hughes-Fulford/ UCSF	Validation of MDA's for coverslips
Mouse bone marrow	M. Lewis/ B. Lawless/ Rockefeller Univ.	Validation of MDA, cell viability
Amphibian cells (Xenopus)	R. Gruener / U. Arizona	Validation of MDA's for coverslips
Algae (Diatoms)	P. Nerren / UAH	Student experiment data obtained
Copper sulfate	J. Baird/ UAH	Fluids mixing in low-g data

**STS-52 CMIX-1 Payload (October 1992):** The first flight of the Commercial MDA ITA Experiments (CMIX) Program Payload was accomplished in October 1992 on STS-52. Table 3 lists ITA commercial users and Student Space Education Program experiments, UAH CMDS affiliates and experiments, and describes experiment rationale and potential commercial applications for the CMIX-1 Payload. Additional UAH CMDS experiments not listed in Table 3 are shown in Table 4.

Table 3. CMIX-1 Experiments Conducted in ITA's MDA Minilab Units on STS-52.



Instrumentation for Material Development in Space  
The University of Alabama in Huntsville  
Huntsville, Alabama 35894-0001  
Phone: (205) 856-4649  
Fax: (205) 856-6791

# CMIX-1 Experiments Conducted in ITA's MDA Minilab Units

INSTRUMENTATION  
TECNOLOGY  
ASSOCIATES, INC.  
ITA

## ITA COMMERCIAL USERS

Experiment Name	PI	Sponsor/Organization	Experiment Rationale / Potential Commercial Applications
1. Urchins Protein Crystal Growth	Dr. Dennis Morrison	ITA/NASA/University of Colorado, Boulder	Grow crystals of urchins to help design a combat spread of breast, brain, and other cancers
2. Collagen/Polyester Alloy Production	Dr. Robert P. O'Fee	Kennedy-Nash, Eton, PA	Determine what new polymer designs have been space processing for future medical products applications. Unusual polymer alloys are sought
3. Inorganic Assembly	Dr. Robert Shaw	Environmental Technology & Telecommunications (ET&T), NY	Inorganic assembly studies
4. Bacterial Alkalase Crystal Growth	Dr. Jürgen Oppenich	University of Montreal, Canada	Determine 3-D structure of alkalase for understanding of enzyme catalysis. Studies may allow understanding of genetic diseases associated with defects in the structure of alkalases
5. Rabbit Muscle Alkalase Crystal Growth	Dr. Jürgen Oppenich	University of Montreal, Canada	Enzyme behavior in microgravity for use in sensors and biocatalysis
6. Enzyme Assembly	Dr. Brad Thompson	Alberta Research Council, Edmonton, Alberta, Canada/ET&T	Obtain adequate crystals to determine how the enzyme interacts with DNA
7. Methyrase Protein Crystal Growth	Dr. Paul Todd	University of Colorado, Boulder/ITA	Maximum size crystals for study of water structure
8. Lysozyme Protein Crystal Growth	Dr. Paul Todd	University of Colorado, Boulder/ITA	Obtain adequate crystals to determine 3-D structure. Commercial applications for cell pharmacology
9. RNA Helase Protein Crystal Growth	Dr. Paul Todd / J. Kim	University of Colorado, Boulder/University of Wisconsin-Parkside	Obtain diffraction quality crystals
10. DNA Helase Protein Crystal Growth	Dr. Paul Todd / J. Kim	University of Colorado, Boulder/University of Wisconsin-Parkside	Obtain data on liquid liquid diffusion and magnetic mixing rates. Verify proper function of MDA units.
11. Myoglobin Protein Crystal Growth	Dr. Paul Todd / J. Kim	University of Colorado, Boulder/ITA	Diffusion rates/quality control of apparatus function
12. Dye Diffusion/Osmotic Mixing	Dr. Paul Todd	University of Colorado, Boulder, Colorado/ITA	Diffusion rates/quality control of apparatus function. Applications in environmental sciences
13. Yeast Cell Diffusion	Dr. Paul Todd	University of Colorado, Boulder, Colorado	Direct measure of microbial cell transport. Applications in environmental sciences
14. Cell Research	Mr. Bill Vandam	Huntsville, AL	Isolation of process control of fluids & cell mixing. Applications for cell culturing on space station
15. Inorganic Assembly	Ms. Valerie Casanova	ITA	Inorganic assembly studies
16. Engineering Tests	Mr. John Casanova	ITA	Obtain data on liquid liquid diffusion and magnetic mixing rates. Verify proper function of MDA units
17. Engineering Tests	Mr. Ted McFarlane, William Dunsen, Mr. Robert Orrill	ITA	Obtain data on liquid liquid diffusion and magnetic mixing rates. Verify proper function of MDA units

## ITA STUDENT SPACE EDUCATION PROGRAM

18. Brassica Rape Reproduction	Ms. Sue Walker	School District of Lancaster, PA for J.P. McCaskey High School	Seeds and reproductive tissue will be grown to propagate successive generations of the plant on Earth. Assessments on any long term effects on hereditary patterns will be made.
19. Mustard Sprout Seed Germination	Mr. Robert Carter	STS-52C Astronaut, New Jersey Schools	Compare space grown sprouts with earth grown. Compare data with similar Shuttle experiments.
20. Cloning Fibroblast Cells/Gels Vessels	Mr. David Brandt, Ms. Angela Mitchell	National Space Society (NSS)/National Capital Space Society for Washington DC and Fairfax, Virginia high schools	Attempt to clone human fibroblast with a replace enzyme found in snake venom. Possible applications: fast acting antibiotics and inoculation to this venom. Gain knowledge on cloning
21. Gene Shrimp Development	Mr. Ramon Nassar, Mr. Scott McCord	Spaceport Florida Authority, Cape Canaveral, FL for Titusville High School	Attempt to hatch tiny Artemia Salina shrimp eggs to determine how microgravity effects early development. The hatchlings will be observed and compared to ground specimens
22. Coseople Seed Germination	Mr. Jim Ralph, Mr. Randall Keigler, Ms. Sue Hutchins, and Ms. Blayden Coughlin	Spaceport Florida Authority, Cape Canaveral for Florida schools	Germinate seeds of Florida's official wildflower, the Coseople. Seeds will be compared with earth bound control seeds
23. Tin Crystal Production	Mr. Barry Feltman	Clute High School, Walunda Middle School, Tase High School	Obtain micro crystals of pure tin metal and compare them with earth grown crystals (a test)

## UAH/CMS AND NASA CENTERS FOR THE COMMERCIAL DEVELOPMENT OF SPACE (CCDS) EXPERIMENTS

24. Bone Cell Function in Microgravity	Dr. M.L. Lewis, Dr. M. Hughes-Fillard	Conducting for Materials Development in Space (CMDS) at the University of Alabama, Huntsville, Alabama (UAH)	Evaluate collagen deposition and cell growth in low gravity in order to gain a database on potential areas for drug remediation of osteoporosis to enhance bone cell growth and prevention of bone deterioration during long-term spaceflight and in the elderly.
25. Phagocytosis	Dr. M.L. Lewis, Dr. B.D. Lawless	UICSF, Veterans Administration Medical Center	Evaluate phagocytic function, the body's defense of ridging based of invading organisms, such as bacteria, leading to a new understanding of cell function for optimized response in microgravity.
26. Immune Cell Response	Dr. M.L. Lewis, Dr. Gary Hall, Dr. David Samone	CMDS at UAH, Rodenhiser University, New York	Gain information on why some cells are more sensitive to gravity than others, that will lead to development of drugs to control undesirable low gravity effects and for Earth based applications
27. Nerve/Muscle Cell Interactions	Dr. M.L. Lewis, Dr. Raphael Guener	CMDS at UAH, University of Iowa, University of Arizona	Model the effects of microgravity on embryonic development of neuron to neuron communication essential for brain function to determine capability for neural brain development in space.
28. Fluids diffusion, magnetic mixing, vital capillary assembly, investigation development, bone development, microencapsulation, tissue structure, and surface solidification	Dr. Brian Spooner, Dr. Jim Coleman	CMDS at UAH, University of Arizona	Various experiments leading to database acquisition for potential commercial processes and services using cell model systems.
29. Signal transduction mechanisms	Dr. Lester Binder	Kansas State University (Bioscience CCDS)	Study low gravity effects on structural components of cells to gain database on cellular mechanisms of responses to microgravity leading to an eventual application such as drug effectiveness screening in low gravity

Table 4. Additional UAH CMDS Experiments flown on STS-52 not listed in Table 3.

Discipline	Experiment	Potential Commercial Application	Investigator Affiliation
<b>Bioprocessing Module Experiments</b>			
Immunology	T lymphocyte activation	Therapeutics	D. Morrison, NASA/JSC Houston
Immunology	T lymphocyte different	Therapeutics	M. Lewis, UAH
<b>Additional MDA Experiments not listed in Table 3.</b>			
Cell differentiation	Bone marrow cell growth	Transplantation	B. Lawless, Rockefeller U. NY
Oxygen regen.	Algae/diatom growth	Closed life support	P. Nerren, UAH
Fluid dynamics	Diffusion	Materials processing	J. Baird, UAH

Four MDA's were flown in the Commercial Refrigerator/Incubator Module (C-RIM); two were filled with CMDS investigators and affiliates and two contained ITA's commercial customer samples. Table 5 lists the CMIX-1 hardware flight configuration.



## FLIGHT CONFIGURATION ON CMIX-1, STS-52

- Four MDA units plus controller and CRIM carrier in CRIM\* at 20°C
  - \* Controller provides power and commands to operate MDA units
- MDA units are qualified for 2 levels of containment
- MDA-4 will be heated to 37°C and be in a lexan 3rd containment vessel for UAH cell research experiments
- MDA controller is velcroed to CRIM carrier for easy removal of BioProcessing Modules (BPM's)
- MDA's 1, 2, and 3 have the capability for manual override contingency on-orbit operations
- The four MDA units will provide more than 300 separate data points on STS-52

Table 5. Flight Hardware Configuration on CMIX-1.



Unfortunately, due to an elevated temperature in the carrier (the NASA provided CRIM), a temperature excursion occurred causing an overtemp by several degrees during the first days of the CMIX-1 Payload operations. Because of this anomaly, much of the data obtained from this flight were inconclusive. Post-flight analyses determined that the higher than acceptable temperature was due to a derating of the CRIM fan to run 50% slower in order to meet Shuttle acoustic requirements. In addition, the CMIX-1 Payload was placed near the galley and water heater which appear to generate significant heat to proximally located Shuttle middeck payloads. Modifications to the CRIM and location of the CMIX Payload far away from the galley and water heater were beneficial results of the lessons learned from this first mission. These modifications were incorporated in the second flight of CMIX on STS-56.

Ten BPMs, (Figure-4) were also flown on STS-52. The BPMs, removed from the CRIM and attached by Velcro to a beta cloth bag placed on the flight deck, were maintained at ambient Shuttle temperature. The BPM experiment, in collaboration with Dennis Morrison, NASA/Johnson Space Center, included human and mouse lymphocytes mixed with different growth mediators and fixed at selected times during flight. The cells survived with viabilities of 70-80% and provided information for the design of the subsequent CMIX-2 Payload.

#### STS-56 CMIX-2 Payload April (1993):

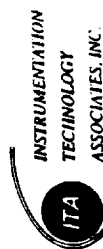
CMIX-2 was essentially a repeat of the CMIX-1 Payload. Tables 7 and 7a list experiments conducted in the ITA MDA Minilabs. The payload was launched on STS-56 on April 8, 1993 and returned after a nominal nine-day mission. CMIX-2 carried four MDA's and consisted of 35 separate experiments (25 commercial or student-affiliated investigations for ITA and ten cell biology experiments conducted by UAH CMDS and collaborating investigators). Approximately 400 data points were obtained from the four MDA minilabs.



Figure 9. Astronaut Ken Cockrell activating MDA's.

Figure 9 shows Astronaut Kenneth Cockrell switching the MDA controller to activate CMIX-2 experiments on STS-56.

Table 6. CMIX-2 Experiments Conducted in ITA's MDA Minilab Units.



# CMIX-2 Experiments Conducted in ITA's MDA Minilab Units

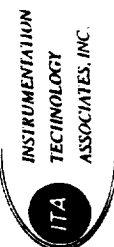


Instrumentation  
Technology  
Associates, Inc.  
For a complete list of experiments in space,  
contact ITA Associates, Inc.,  
P.O. Box 1000,  
Baltimore, MD 21201.

## ITA COMMERCIAL USERS

Item	Experiment Name	PI	Sponsor/Organization	Experiment Rationale / Potential Commercial Applications
1	Collagen Reconstitution	Dr. Kenneth E. Hughes	ITA/NASA, ISC/University of Colorado, Boulder	Studies of fibrin growth in micro g. Applications include unique & complex products for implants
2	Urokinase Protein Crystal Growth	Dr. Dennis Morrison/Mr. John Cassiano/Dr. Paul Todd	ITA/NASA, ISC/University of Colorado, Boulder	Grow crystals of urokinase to help design drugs to combat spread of breast, brain, and other cancers
3	Microencapsulation	Dr. Benjamin Mosser/Dr. Dennis Morrison	Institute for Research, Houston, Texas	Microencapsulation of drugs for drug delivery techniques. Applications to improve chemotherapy drug delivery, to encapsulate inhalant medications, enhance radiographic procedures
4	Inorganic Assembly	Dr. Robert Shaw/Ms. Ann Deering	Environmental Technology & Telecommunications (ET&T), NY	Inorganic assembly studies
5	Bacterial Aldolase Crystal Growth	Dr. Jürgen Sygusch	University of Montreal, Canada	Determine 3-D structure of aldolase for understanding of enzyme catalysis. Studies may allow understanding of genetic defects associated with defects in the structure of aldolase
6	Rabbit Muscle Aldolase Crystal Growth	Dr. Jürgen Sygusch	University of Montreal, Canada	Attempt to crystallize this protein. Applications for AIDS research
7	HIV Reverse Transcriptase	Dr. Paul Todd/Dr. Richard Konstan/Mr. John Cassiano	University of Colorado, Boulder/ITA, Etion, PA	Obtain adequate crystals to determine how this enzyme interacts with DNA
8	Methylase Protein Crystal Growth	Dr. Paul Todd/Dr. Richard Konstan	University of Colorado, Boulder/ITA	Maximum size crystals are sought for study of water structure
9	Lyszyme Protein Crystal Growth	Dr. Paul Todd/Dr. Richard Konstan	University of Colorado, Boulder/ITA	Obtain data on liquid liquid diffusant and magnetic mixing rates. Verify proper function of MDA units
10	Myoglobin Protein Crystal Growth	Dr. Paul Todd/Dr. Richard Konstan	University of Colorado, Boulder/ITA	Diffusion rate/quality control of apparatus function/commercial microbiology in spaceflight
11	Dye Diffusion/Magnetic Mixing	Dr. Paul Todd	University of Colorado, Boulder/ITA	Direct measure of microbial cell transport. Applications in environmental sciences
12	Yeast Cell Diffusion	Dr. Paul Todd	University of Colorado, Boulder/ITA	Diffusion rate/quality control of apparatus function
13	Human Serum Albumin Phosphatase	Dr. David Barford	Cold Spring Harbor Laboratory, Cold Spring Harbor, New York	Determine 3D structure of this enzyme
14	Human T cell Iysozyme phosphatase	Dr. David Barford	Cold Spring Harbor Laboratory, Cold Spring Harbor, New York	Determine the 3D structure of the key immune system enzyme
15	Cell Research	Mr. Bill Vardaman/Dr. Marian Lewis	Rutich, Huntsville, AL	Exploration of process control of fluids & cell mixing. Applications for cell culturing on space station
16	Bone Shrimp Development	Ms. Valerie Cassiano/Mr. Ulfes Alvarado	ITA, Etion, PA	Success in hatching bone shrimp eggs in the MDA. Potential for shrimp as space food source
17	Inorganic Assembly	Mr. Ulfes Alvarado/Ms. Valerie Cassiano	ITA, Etion, PA	Inorganic assembly studies
18	Engineering Tools	Mr. John Cassiano/Mr. Ulfes Alvarado	ITA, Etion, PA	Obtain data on liquid liquid diffusion. Verify proper function of MDA units
19	Magnetic Mixing Tools	Mr. Ted Muller/Mr. William Dunfee/Mr. Robert Oskul	ITA, Etion, PA	Obtain data on liquid liquid diffusion and magnetic mixing rates. Verify proper function of MDA units

Table 6a. CMIX-2 Experiments (continued).



## CMIX-2 Experiments Conducted in ITA's MDA Minilab Units



Consortium for Materials  
Development in Space  
The University of Alabama at Huntsville  
Huntsville, Alabama 35894  
Tel. 256/890-6200

### ITA STUDENT SPACE EDUCATION PROGRAM

20	Brassica Rape Reproduction	Ms. Sue Walker, Mr. David E. Harsh	School District of Lancaster, PA (or J.P. McCaskey High School)	Seeds and reproductive tissue will be flown to propagate successive generations of the plant on Earth. Assessments on any long term effects on hereditary patterns will be made.
21	Fish Egg Hatching	Mr. Charles A. Roney	Pennville Memorial High School, Pennsville, New Jersey	Attempt to hatch African fish eggs in space
22	Fish Egg Hatching	Mr. Eddie Ellegood, Mr. Eugene Hull	Spacelab Florida, ORBIS Scientific, Florida	Determine effect of micro g. on morphology rate of heart muscle cells
23	Heart Cells in Culture	Mr. Robert Carter, Mr. Ned Bean, Mr. Alan B. Rynne, E. Crockett, T. Cechowski, J. Healy, A. Kohn, R. Robertson, L. Worthington, M. Zales	STS-61C Astronaut, the Peddie School, New Jersey	Mushroom mycelium will be flown in microgravity for comparison and eventual growth of new and improved mushrooms
24	Mushroom Mycelial Growth	Ms. Helen E. Martin, Mr. Heather D. Suter, Mr. Bart Brutscher, Mr. Bob Parnell, Mr. John B. Swaine, Ms. Sheryl L. Harris, Mr. Douglas A. Dente	Hawthorne High School, John B. Swaine Sprawl Company	A variety of dry seeds will be flown such as radish, lettuce, tomato, and pea. Proper changes in blood cell shape will be studied after experiencing the effects of microgravity.
25	Seeds in space	Bob Mohler	Lockheed	
26	Human Red Blood Cell Morphology	Mr. Peter W. Wood, Dr. John M. Wallman	International Space University (ISU)	

### UAH/CMDS AND NASA CENTERS FOR THE COMMERCIAL DEVELOPMENT OF SPACE (CCDS) EXPERIMENTS

27	Bone Cell Differentiation	Dr. M.L. Lewis, Dr. M. Hughes-Fulford	Consortium for Materials Development in Space (CMDS) at the University of Alabama, Huntsville, Alabama (UAH), UCSE, Veterans Administration Medical Center	Experiments treatment screening of cellular level responses to drugs in micro g. Potential application is in remediation of space travel bone loss and in the treatment of Earth based osteoporosis in the elderly (Pharmaceutical Industry)
28	Hematopoietic Stem Cell Expansion	Dr. M.L. Lewis, Dr. B.D. Lawless	CMDS at UAH, Rockefeller University, New York	Reconstitution of immune system after radiation or chemotherapy. Applicable in treatment of leukemia, lymphoma, breast cancer. Applications to bone marrow transplantation. Projected Earth based market estimated at several billion dollars annually
29	Immature Cell Response	Dr. M.L. Lewis, Dr. Gary Noll, M.D.	CMDS at UAH, University of Iowa	Production of cytokines, and selected cell types for potential use in treatment of immune suppressed patients. Screening of modulators of immune response in micro g. for overvital use in individuals who experience long term low g.
30	Nerve/muscle Cell Interactions	Dr. M.L. Lewis, Dr. Raphael Guener	CMDS at UAH, University of Arizona	Micro g. may alter some molecular mechanisms in the formation of the neuromuscular junction. Amphibian cells (frog) are being used to model neuro muscular junction formation at the cellular level. This is primarily a database expansion experiment
31	Hard diffusion, magnetic mixing, viral capsid self assembly, invertebrate development, bone development, microencapsulation, dation structure, and surface siliconization	Dr. Brian Spooner Dr. Jan Guikema	Huachuca State University	Various experiments leading to database acquisition for potential commercial processes and services using cell model systems
32	Isotations	Mr. Philip H. Hirsch	CMDS at UAH	Qualants (siliconized wafers) are being flown to investigate their potential use as a component of regenerative life support systems for oxygen production. The objective will be to determine survival, colonization and structure of two species of diatoms
33	Fluid Dynamics	Dr. James Baird	CMDS at UAH	In the absence of sedimentation and convective mixing in microgravity, fluids behave different than in unit gravity. This is the basis of unique fluid dynamics processes which may be conducted only in microgravity

The objectives of UAH CMDS CMIX-2 investigations on mouse, human, amphibian and algal cell cultures, and ITA non-proprietary investigators on collagen assembly, seed germination, microencapsulation, fluid dynamics and protein crystal growth were to expand knowledge of low-g response and to identify potential processes and products which can benefit form space bioprocessing. One MDA was adapted for cells on coverslips, 37°C incubation and triple containment. Three MDA's operated at 20°C for plant and amphibian cells, protein crystal growth and other bioprocessing experiments.

Significant information was obtained on mechanisms of bone and immune cell growth and function. Altered cytoskeletal morphology in both mammalian (Figure 10) and amphibian cells (Figure 11) confirmed cytoskeletal sensitivity to gravity.

In collaboration with Millie Hughes-Fulford, we showed that the actin cytoskeleton of the flown mouse osteoblasts appears bunched and the cell shape is drastically different from the ground control. This data indicates that the cytoskeleton in these cells is extremely gravity sensitive. Implications are that cellular function may also be drastically affected in low-g. We also confirmed results of Skylab experiments which reported reduction in glucose use during spaceflight. Our future experiments will utilize this mouse bone cell model system to test compounds supplied by a leading pharmaceutical company which are designed to prevent or control space osteoporosis.

Similar results were obtained with the *Xenopus* muscle cells of Raphael Gruener. Flown cells showed decreased actin filament linearity and increased actin filament "cabling" as shown in Figure 11. These data are interpreted to mean that the cytoskeleton may be involved in grav-sensing by single cells.

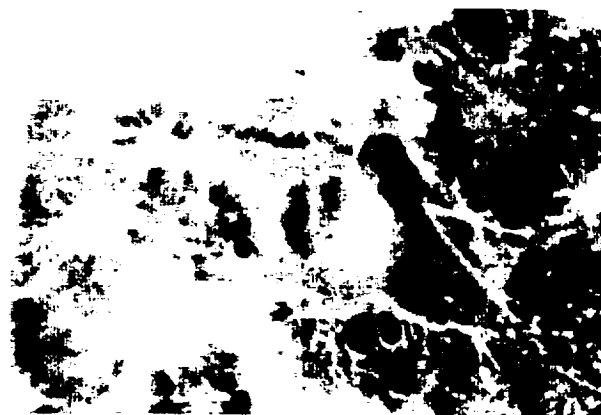
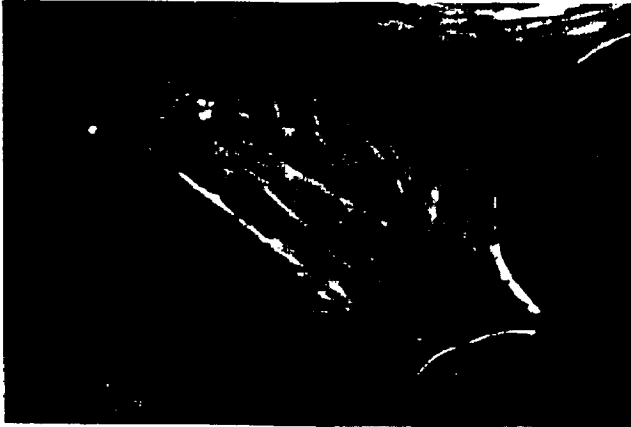


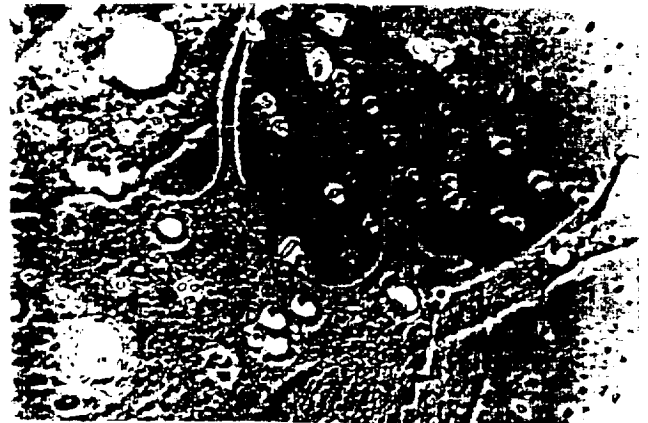
Figure 10. Mouse bone 3T3 cells grown on coverslips in the MDA wells in ground-based (Top) and flight (STS-56) (Bottom). (Photographs, provided by Dr. Millie Hughes-Fulford, UCSF).

In typical ground control tests, frog cells utilize yolk platelets as a source of energy as they grow. The flown cells were smaller in size and had an over-abundance of yolk platelets indicating a significant slowing of development in space flown cells (Figure 12). These data imply significantly altered development on nerve and muscle cells in low-g and may serve as a model for drug testing and cellular mechanism definition.

**Figure 11.** Xenopus actin cytoskeleton of control (Top) and flown cells (Bottom)



**Figure 12.** Xenopus cells from ground (Top) and flown cells (Bottom).



(Xenopus photographs courtesy of Dr. Raphael Gruener, University of Arizona, Tucson, AZ)

Human lymphocytes flown in the BPM's maintained high viability (Figure 13) but the flown cells did not increase in number (Figure 14) and were shown by flow cytometry to remain mostly in a non-growth state, whereas the ground control cells responded to mitogenic stimulation and increased in number. In a related experiment using the MDA with mouse T lymphocytes in collaboration with Dr. Mary Ann Principato of the Food and Drug Administration, we determined that the T-cell receptor is not affected by low-g, however; non-T cell mediated activation (Con-A) is severely blunted in low- gravity.

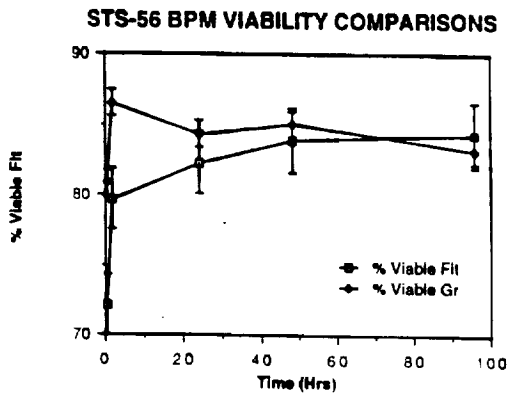


Figure 13. Viability of human T lymphocytes in the Bioprocessing Modules on STS-56. Viabilities of flight and ground cells were approximately the same during the test.

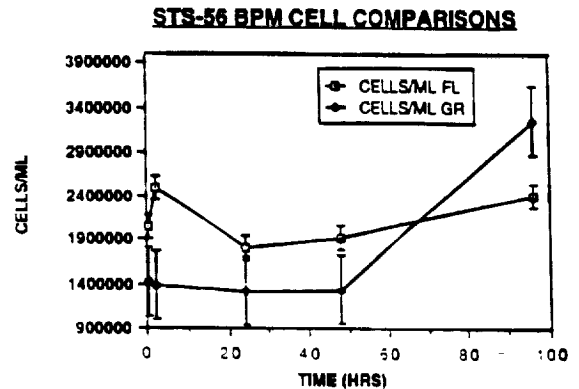


Figure 14. Counts of human T lymphocytes flown in the BPM's on STS-56. Ground control counts were significantly higher than flown counts after four days.

Using the ITA MDA we have thus defined a tool for the study of mechanisms of growth regulation and can now begin to manipulate growth factors and mediators to expand the database on cellular signaling. This can be important to research in the area of cancer and other immune cell disease states.

The CRIM hardware functioned well on CMIX- 2. The temperature remained within acceptable limits as shown by plots obtained from data from the thermistors placed at several locations within the CRIM (Figure 15 a,b,c,d thin line plots). Thermistor data indicated that MDAs 1, 2, and 3 operated below 23°C during the first four days and dropped back to 20°C for the remainder of the mission. MDA-4 maintained the set 37°C for the four day cell tests then dropped back to 20°C as planned.

The CMIX-2 payload demonstrated low-cost basic cell biology and bioprocessing research and potential for manufacturing in space using the generic multipurpose commercially-developed MDA hardware.

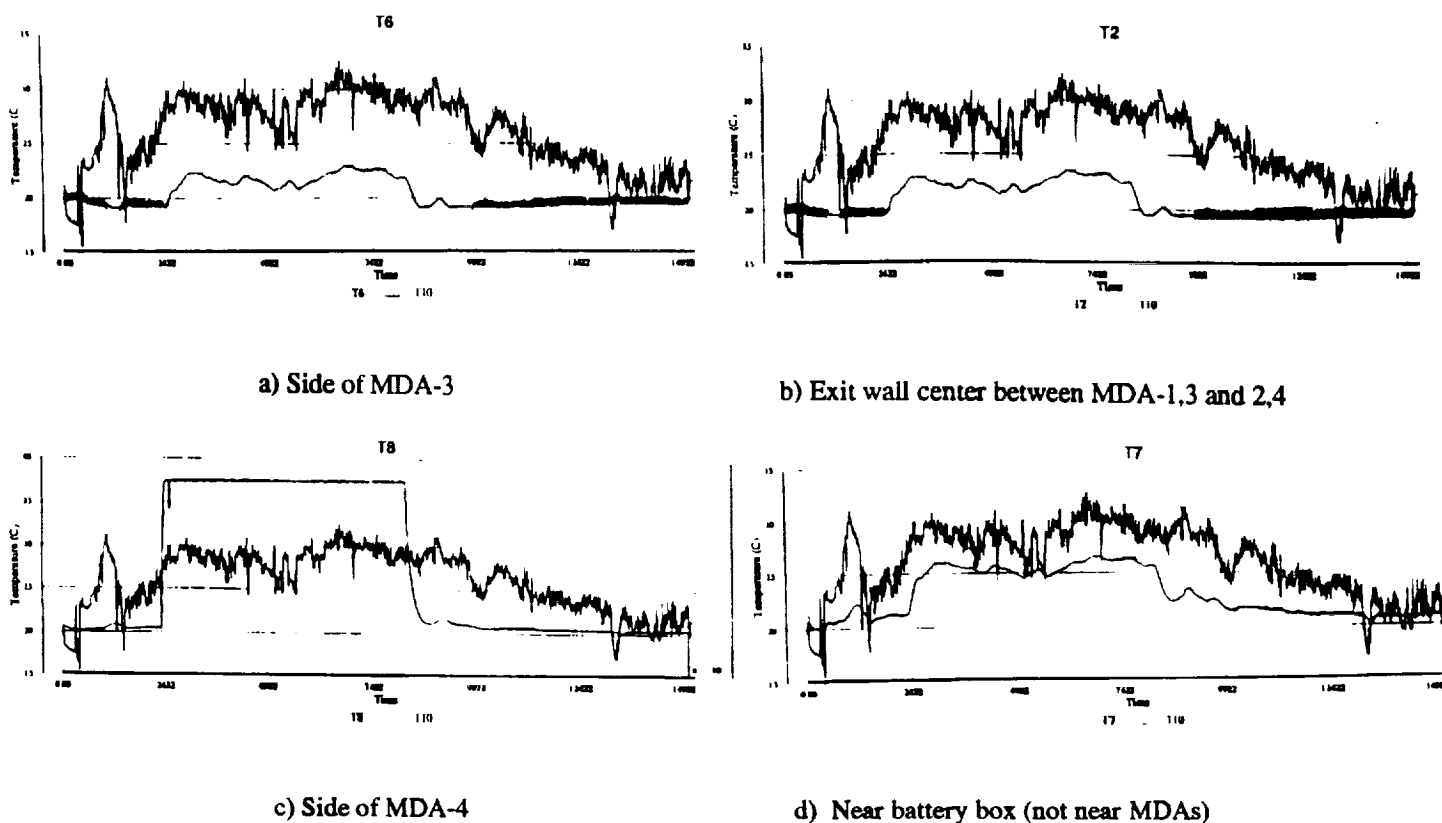


Figure 15. Temperature profiles of data from thermistors located at various places inside of the CRIM.

### **BIODYNAMIC CELL CULTURE SYSTEMS (ROTATORY CULTURE BIOREACTOR)**

This project has both a ground-based component and a microgravity capability. In theory, the microgravity environment of space is expected to offer a unique advantage to the culture of anchorage dependent cells growing on microcarrier beads in that no agitation will be required to keep cells suspended. Gentle agitation can be carefully controlled to ensure that oxygen and nutrients are accessible to cells and metabolic products are removed from the micro-environment. Thus, microgravity can be exploited as a way to increase the aggregation of cells of similar or different types into tissue-density three-dimensional associations. In addition to growth of anchorage dependent cells, microgravity may offer a very unique advantage to suspension cultured cells by providing an environment in which selected cell types prosper and others do not differentiate and grow. The reduced response of T-lymphocytes to mitogens in microgravity is an

example. Because of this differential response of cells in microgravity, new ways to separate desirable cell types may be exploited. This project is maturing rapidly and hardware can be ready for flight by the first or second quarter of 1994.

The ground-based rotating culture devices used by this project produce a very low-shear culture environment and appear to simulate some aspects of microgravity in that they allow cells to remain evenly suspended in three dimensional configuration in a gentle culture environment similar to that expected in microgravity. Low shear culture is achieved and microgravity simulation is approached by randomizing the gravity vector. Rotating culture systems such as these are being used in the Bioreactor Laboratory at UAH for investigating the effect of altered gravity on both plant and animal cells.

**THE APPARATUS** (Available through Synthecon, Inc. Friendswood, TX)

The rotary cell culture bioreactor systems (RCCS) are simple, motor driven, non-perfused 100, 250 or 500 ml volume, horizontally oriented cylindrical vessels which rotate around the horizontal axis and operate in a standard CO<sub>2</sub> incubator. (Figure 16).

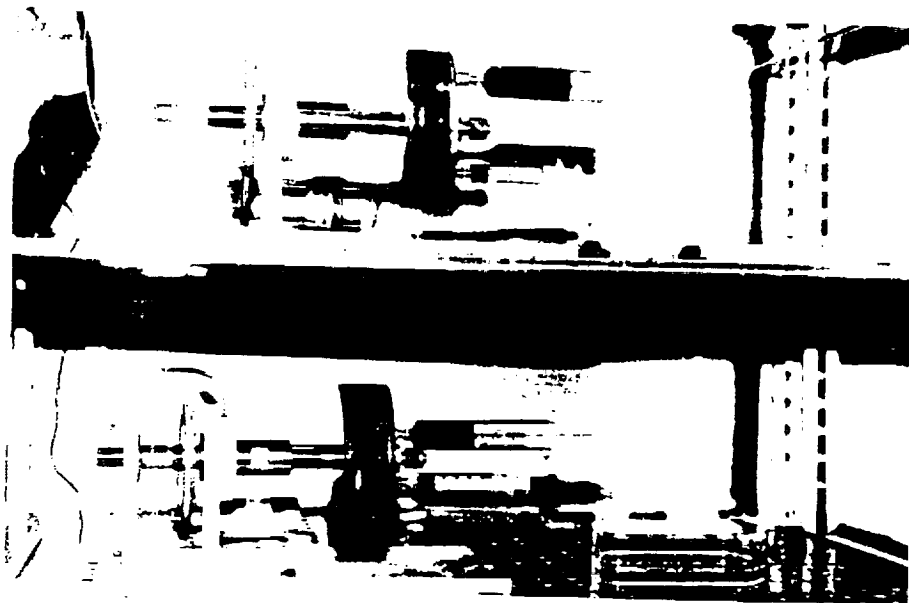


Figure 16. Two High Aspect Rotary Cell Culture Systems operating in the CO<sub>2</sub> incubator at UAH.

Rotation rate is adjustable from 0 to 75 rpm. Cells growing in the vessel are oxygenated by pumping 95% air-5% CO<sub>2</sub> through a central shaft covered with a gas exchange membrane or by passive air exchange over a larger membrane covering one end of the vessel. Vessel rotation at 8



to 20 rpm is sufficient to keep cells or cell-bead aggregates uniformly suspended during the early culture phase. As aggregates become larger as in the case of microcarrier cultures, the rotation rate is increased to maintain a uniform suspension. For cells growing on microcarrier beads, nutrients are replenished by allowing beads to settle to the bottom of the vessel, removing spent medium and replacing it with fresh medium. For suspension cells such as lymphocytes, the contents of the vessel are removed, cells are spun out, suspended in fresh medium and reloaded into the culture vessel. A transient disruption of the quiescent culture environment occurs during medium changes.

Flight model bioreactors will be ready to fly in the first or second quarter of 1994. The flight model will be an on-demand perfusion system in which a high aspect vessel is equipped with a chamber containing fresh culture medium. This medium chamber is separated from the cell culture chamber by a defined molecular weight cutoff membrane. High density cultures have been grown successfully on the ground in this device, the DHARV (Diffusion or Double High Aspect Rotating Vessel), a modification of the RCCS.

We have used this culture system to demonstrate that mouse 3T3 osteoblasts grow in three-dimensional aggregates and produce collagen in the matrix between the microcarrier beads (Reported at the 1991 ASGSB meeting in Louisville). Lymphocytes exposed to anti-OKT3 in the presence of IL2 differentiated and secreted cytokines into the medium. We are continuing to use the RCCS for three-dimensional modeling and mechanism research.

## CONCLUSIONS

This funding supported, in part, experiments conducted on two Consort sounding rockets and five Shuttle flights. The achievements described in this report were the result of collaborative research among selected investigators brought into the collaboration for the purpose of gaining specific microgravity effect information. We (Hughes-Fulford, Gruener, Lewis) showed that the cytoskeleton is altered in low-g. Implications are that any cellular function, including signal transduction and secretory processes, which depend on the cytoskeleton may be affected during spaceflight. We intend to continue this research to fine tune the mechanisms of how microgravity influences molecular assembly. We (Principato, Lawless, Ochoa, Lewis) confirmed that the T cell receptor-mediated signal transduction pathway in T lymphocyte activation is not affected by low-gravity compared to non-TCR-mediated stimulation (Con-A) which classically does not promote proliferative response. This implies that microgravity may allow separation of proliferative signaling and secretory function in lymphocytes. To use microgravity in this way can permit us to specifically select mediators to put cells into either a proliferative or secretory state. Basic research and commercial production of selected cytokines are potential applications for this finding. The

demonstration of increased prostaglandin synthesis (Hughes-Fulford) during reduced bone cell growth, suggesting an effect of microgravity on prostaglandin-induced mitogenesis, can allow us to investigate the mechanisms controlling bone cell growth in low-g.

The funding contributed significantly to the scientific database in the areas described. We have three, and possibly four, Shuttle flights remaining in the CMDS CMIX Program extending through 1996 or 1997. We will also have about one sounding rocket launch per year. We hope to obtain funding from NASA Life Sciences to allow us to continue this extremely productive collaborative research and maximize our low-g access.

### PUBLICATIONS AND PRESENTATIONS

1. Lewis, M.L. "Cellular Responses to Low-g". Invited seminar, Kansas State University, Manhattan, Kansas, Sept, 1991.
2. Lewis, M.L., M. Kumegawa, S. Doty, and Millie Hughes-Fulford. Characterization of bone cell growth and differentiation in multi-dimensional culture. American Society for Gravitational and Space Biology, October, 1991.
3. Conway, Rebecca, Adriel Johnson and Marian Lewis. Effects of microgravity on pancreatic cells. Alabama Academy of Science 62: 74. (1991)
4. Lewis, Marian L. Materials Dispersion and Biodynamics Project. First Annual Space Station Freedom Utilization Conference Proceedings, August 3-6, 1992.
5. Chapes, S.K., D.R. Morrison, J.A. Guikema, M.L. Lewis, and B. S. Spooner. "Cytokine production by immune cells in space. J. Leukocyte Biol. 52:104-110 (1992).
6. Cassanto, John R. and Marian L. Lewis. Commercial Materials Dispersion Apparatus on the Space Shuttle. IAF section of the World Space Congress, Washington D.C., August 29-Sept. 5, 1992.
7. Lewis, Marian L. and B. DeSales Lawless. Results of STS-43 Cell tests in the Materials Dispersion Apparatus (MDA). American Society for Gravitational and Space Biology, Tucson, AZ. Poster # 50. October 20-24, 1992.
8. Johnson, A.D., R.P. Conway, C.A. Meacham and M.L. Lewis. Effect of a low-shear horizontally rotating culture system on embryonic chick pancreatic tissue function. American Society for Gravitational and Space Biology, Tucson, AZ, Oct 20-14, 1992.
9. Conway, Rebecca, Connie Meacham, Adriel Johnson and Marian L. Lewis. Effects of a unique rotating culture system on pancreatic release. Alabama Academy of Science. 1992.

Abstracts submitted to the 1993 Annual Meeting of the American Society for Gravitational and Space Biology (ASGSB) based on results from CMIX-1 (STS-52) and CMIX-2 (STS-56).

1. M.L. Lewis, and J.M Cassanto. Automated, multiple-sample minilabs provide significant cell biology and space bioprocessing data for Shuttle middeck research.
2. M. Hughes-Fulford, K. Nelson, S. Blaug, C.G. Summer, B.D. Lukefahr and M.L. Lewis. MC3T3-E1 osteoblasts grown in microgravity on STS-56 have reduced cell growth, glucose utilization with altered actin cytoskeleton and increased prostaglandin synthesis.

3. G.A. Neil, L. A. Love-Homan, C. Summer, D.W. Sammons and M.L. Lewis, Human splenic B cell activation *in vitro* is not impaired by microgravity (ug).
4. R. Gruener, R. Roberts and R. Reitstetter. Exposure to microgravity alters properties of cultured muscle cells.
5. M.A. Principato, B.D. Lawless and M.L. Lewis. Superantigen-mediated T cell receptor stimulation in zero gravity.

**Abstract submitted to the 1993 American Society for Cell Biology (ASCB) Annual Meeting.**

6. M.L. Lewis, M.A. Principato, B.D. Lawless, D.R. Morrision, W.C. Kapp, S.L. Strobl and A.C. Ochoa. Effects of microgravity on leukocyte growth and function.

## Local/State

# UAH experiments aboard shuttle

By MARTIN BURKEY  
Times Science Writer

Out of sight but not out of mind during the space shuttle mission this week is a package of experiments from the University of Alabama in Huntsville.

Containing vials of human, frog and mouse cells, the experiments could offer clues to Earth-based ailments, UAH scientists said.

The Commercial MDA ITA experiment (CMIX 02) payload occupies one locker on the shuttle mid-deck. It consists of four brick-sized automated Material Dispersion Apparatuses. They are turned on by the crew and allowed to run until the end of the mission. This week, they are being used to conduct more than 30 experiments.

The devices were developed by Instrumentation Technology Associates Inc. of Exton, Pa.

Principal investigator and CMIX project manager Dr. Marian Lewis, of UAH, said human, mouse and frog cells will be used to learn how the human body responds to living in the weightless environment of space. The research may also give clues toward cures for certain diseases and conditions on Earth.

The research will focus on how cell structure and function are altered in microgravity and could contribute to remedies for some of the physiological problems of space adaptation experienced by astronauts, she said.

The research is sponsored by the UAH Consortium for Materials Development in Space CMDS. The UAH CMDS is one of 17 Centers for the Commercial Development of Space sponsored by NASA's Office of Advanced Con-

## No changes planned in booster bolt device

By MARTIN BURKEY  
Times Science Writer

Marshall Space Flight Center officials said they plan no changes in a shuttle booster restraining device that broke loose during last week's launch of the Discovery.

Each booster has four hold-down posts that fit into support posts on the launch pad. During thrust buildup after ignition, 3-inch-diameter bolts hold the shuttle to the pad. Two detonators then split the nut atop each bolt and allow the

shuttle to lift off.

A container was added to the mechanism after the 1986 Challenger accident to hold any debris that might otherwise get blasted by the booster exhaust and bounce up into the external liquid-fuel-tank booster Project Manager Cary Rutland said.

After the nut breaks, the bolt is supposed to slide down into a bucket of sand, followed by an aluminum plunger to plug the hole through which it was projected in order to keep other debris from dropping down, Rutland said.

The plunger apparently broke free also and fell through the open

hole. It could have bounced into the exhaust path but fortunately landed in the sand bucket, Rutland said.

Evidently, the fractured nut also knocked the lip off the plunger, Rutland said.

"We think it was a freak occurrence," Rutland said. "If it comes out the wrong time and place, it becomes pad debris. With the right plume impingement, it could bounce around and contact the vehicle."

Rutland said the device has worked correctly 29 times since it was added.

cepts and Technology.

Scientists have learned that microgravity causes several changes in the human body. During space travel, astronauts and cosmonauts have experienced bone demineralization, muscle atrophy, cardiovascular deconditioning, reduced immune cell response and even decreased in red blood cell counts, Lewis said.

These space travel-induced conditions correspond to health problems on Earth such as osteoporosis, anemia, immune deficiencies and other types of immune cell dysfunctions.

Lewis said the cell experiments flying on CMIX may give insights into cellular-level mechanisms and contribute to the understanding and treatment of these Earth-based conditions.

These live cell tests on bone cell

development, lymphocyte function and muscle-nerve cell responses will contribute information for potential development and testing of drugs to modify or eliminate some of the undesirable effects of space travel, said Lewis.

Two UAH students also have experiments in the CMIX 2 payload.

Philip Nerren, a graduate student in biology, is flying an experiment with diatoms, single-celled microscopic plants. This study is crucial in evaluating whether diatoms could be used in a biological recycling system for air and water for long-term space missions. The diatoms might help recycle carbon dioxide produced by humans.

Diatoms have a silicon cell wall instead of cellulose. This makes the cells more durable, Nerren said.

The diatoms were dehydrated before launch and rehydrated during the mission and their growth monitored.

Dan Baker, a graduate student in cellular biology, is studying how darkness affects the growth patterns of algae. Algae is ecologically important because it could serve as a food, fertilizer as well as suit industrial and pharmaceutical needs. Several of those uses might be especially useful on a space station or a long space flight.

Other samples in the CMIX payload will study nerve cell communication, the body's mechanism for fighting infection, microencapsulation of drugs, AIDS, heart cells, and germination of mushroom spores and mustard and mustard spinach seeds.

## Discovery crew catches sunrises; pliers embarrass NASA

By SUSAN HIGHTOWER  
The Associated Press

SPACE CENTER, Houston — Discovery's astronauts today twirled the shuttle to catch the dramatic sunrises and sunsets in space and began packing equipment for the trip home.

An embarrassed NASA, meanwhile, chastened after the use of

tools on the launch pad and opened an investigation into how Discovery lifted off with a pair of pliers stuck on a rocket booster.

NASA said the pliers posed no danger during liftoff. But officials were amazed the tool, 8 to 10 inches long, remained wedged in a metal pocket on the outside of one of the twin boosters.

NASA spokeswoman Lisa Malone said a technician for booster maker Thiokol Corp. noticed his

pliers were missing April 2, six days before Discovery blasted off.

The technician told his supervisor, but the supervisor never filed a report and consequently no search was conducted, officials said. Before launch, shuttle managers were unaware the pliers had been lost at the pad, Malone said.

In space, everything continued to go well for Discovery's five crew members on an atmospheric re-

have been taking solar readings and measuring the shrinking ozone layer.

Pilot Stephen Oswald today alternately turned shuttle so its bay faced the glaring sun, then spun it toward deep space to cool instruments taking measurements of solar energy.

Oswald and astronaut Ellen Ochoa also turned off and put



Consortium for Materials  
Development in Space

The University of Alabama in Huntsville  
Huntsville, Alabama 35894  
Phone (205) 895-6870  
Fax (205) 895-6791

# MATERIALS DISPERSION AND BIODYNAMICS PROJECT

## CMIX FLIGHT RESULTS CELL BIOLOGY

### BONE CELLS: Mouse osteoblasts grown on coverslips in the MDA.

Project collaborating scientist, Dr. Millie Hughes-Fulford, Univ. CASF

#### Flight result

- o Cytoskeleton structure altered
- o Glucose use less in microgravity
- o Cellular growth rate retarded
- o Increased prostaglandin synthesis

#### Commercial Importance of this research

We have system to test drugs at the cellular level to prevent space osteoporosis. Benefits to ground-based treatment of osteoporosis possible.

Status: Ready to begin drug testing.  
In negotiation with  
pharmaceutical industry

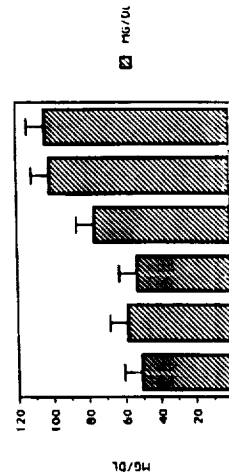
#### Significance

Cell function may be altered  
Metabolism is altered  
Bone replacement reduced  
Bone mitogenesis control

GLUCOSE LEVELS - STS-56  
Mouse 3T3 Osteoblast Cells On Coverslips

COMPARISON OF GLUCOSE UTILIZATION BY FLOWN AND GROUND  
CONTROL SAMPLES  
Table 6

#	DESCRIPTION OF SAMPLES	AVG MG/DL	±	%CV	n
(1)	POST FLIGHT GROUND (TYPE 2 WELLS)	50.17	±2.7	5.4%	6
(2)	POST FLIGHT GROUND (TYPE 3 WELLS)	58.37	±5.8	9.9%	8
(3)	SIMULTANEOUS GROUND CONTROL (TYPE 2 & 3 WELLS)	52.66	±3.9	4.0%	5
(4)	FLIGHT (TYPE 2 & 3 WELLS)	76.80	±9.1	12%	5
(5)	FRESH CULTURE MEDIUM (NO CELLS)	102.0	±1.2	1.1%	5
(6)	CULTURE MEDIUM - ROOM TEMP (NO CELLS)	104.00	±1.0	0.1%	2





Mouse bone 3T3 cells grown on coverslips in the Materials Dispersion Apparatus wells on STS-56. The actin cytoskeleton was stained with rhodamine phalloidin. Cell growth was stimulated with fetal calf serum for four days, then fixed and evaluated by fluorescence microscopy after return of the CMIX-2 Payload.



Top: Actin cytoskeleton of ground control cells run concurrently with the flight experiment. Network of actin filaments are well defined and extend to periphery of the cell.



Bottom: Actin cytoskeleton of cells flown on STS-56. The actin filaments appear bunched and the cell shape is drastically different from the control. This data shows that the cytoskeleton in these cells is extremely gravity sensitive.

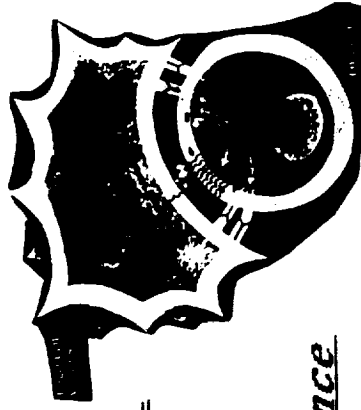


Consortium for Materials  
Development in Space

The University of Alabama in Huntsville  
Huntsville, Alabama 35897  
Phone (205) 895-6670  
Fax (205) 895-8791

## MATERIALS DISPERSION AND BIODYNAMICS PROJECT

### CMIX FLIGHT RESULTS CELL BIOLOGY



#### LYMPHOCYTES (Immune System Cells)

##### Flight result

- o Response to growth mediators variable
- o Glucose use is less in microgravity
- o Cellular growth rate retarded
- o T cell receptor is not affected
- o Non-T-cell receptor response reduced
- o Human B cells response not impaired

##### Significance

Cell signaling altered  
Metabolism is altered  
Other functions may be active  
Immune system function OK  
Research tool (mechanisms)  
Immune system function OK

Commercial Importance of this research  
Results indicate that some cellular products (cytokines) may be produced at higher levels in microgravity. These can be target biopharmaceuticals for low-g production.

Status: Ready to begin communications with pharmaceutical and biotechnology industries interested in immune cell products.



Consortium for Materials  
Development in Space

The University of Alabama in Huntsville  
Huntsville, Alabama 35894  
Phone (205) 885-6670  
Fax (205) 893-6791

INSTRUMENTATION  
TECHNOLOGY  
ASSOCIATES, INC.

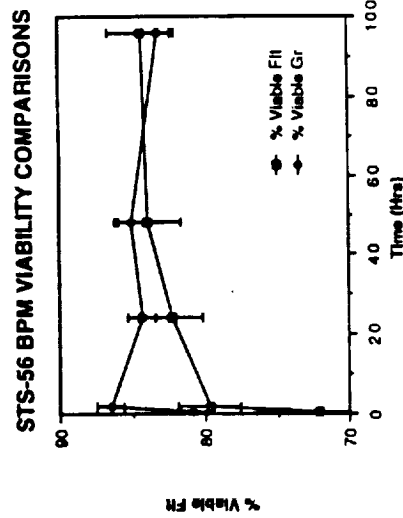
ITA

# MATERIALS DISPERSION AND BIODYNAMICS PROJECT

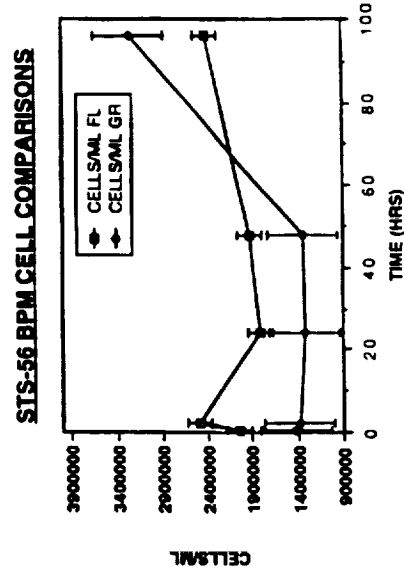
## CMIX FLIGHT RESULTS

### CELL BIOLOGY

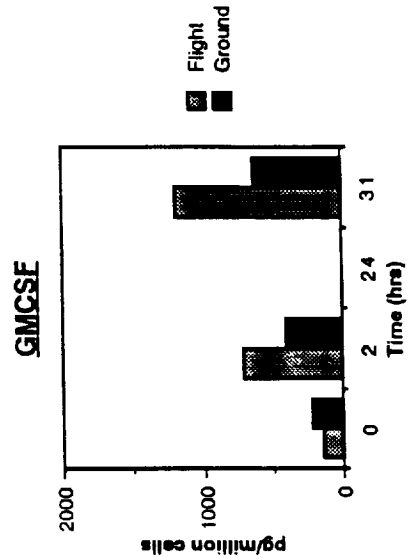
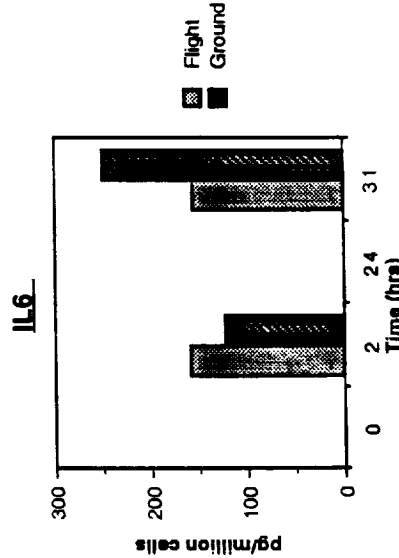
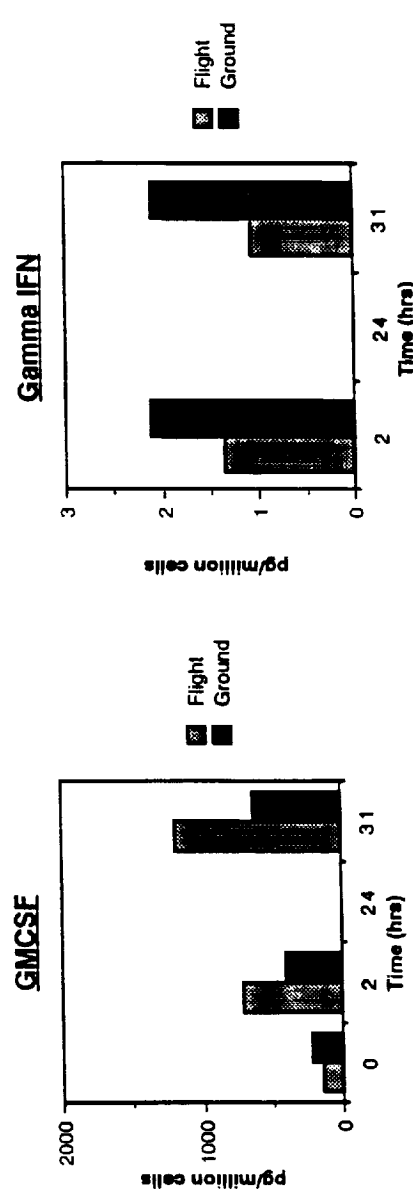
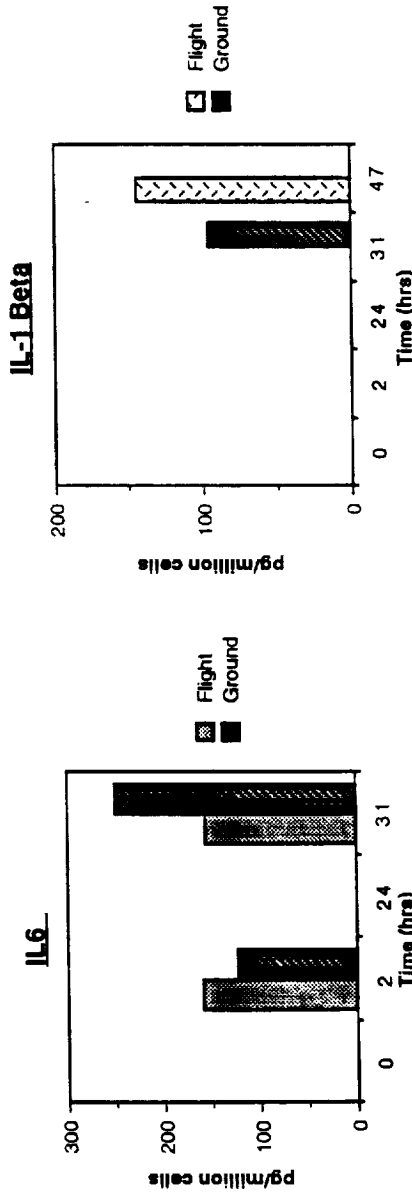
**Figure 20.** Viability of human T lymphocytes in the Bioprocessing Modules on STS-56.



**Figure 21.** Cell counts of human T lymphocytes flown in the BPMs on STS-56. Ground control cell counts were significantly higher the flown counts after four days.



## LYMPHOCYTES (Immune System Cells)







Consortium for Materials  
Development in Space

The University of Alabama in Huntsville  
Huntsville, Alabama 35891  
Phone (205) 895-6670  
Fax (205) 895-6791

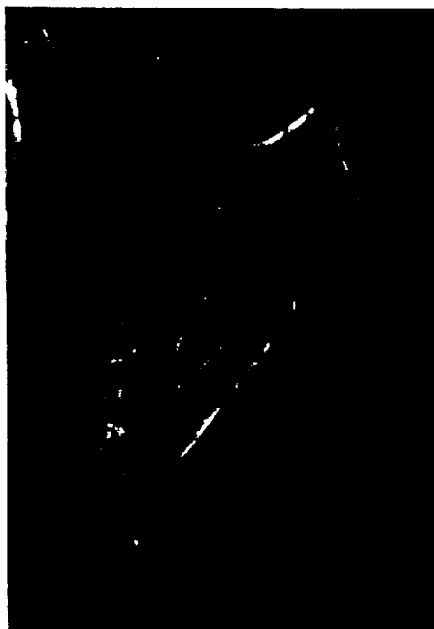
INSTRUMENTATION  
TECHNOLOGY  
ASSOCIATES, INC.

ITA

## MATERIALS DISPERSION AND BIODYNAMICS PROJECT

### CMIX FLIGHT RESULTS CELL BIOLOGY

Amphibian Cells (Xenopus myocytes) Grown on coverslips in the MDA.



Photographs courtesy of Dr. Raphael Gruener, Univ. Arizona

CMDS PEER REVIEW August 18, 1993. Materials Dispersion and Biodynamics Project, M.L. Lewis, Ph.D., Manager.

## **Consortium for Materials Development in Space**

University of Alabama in Huntsville  
Huntsville, Alabama 35899  
Phone: (205) 895-6620  
Fax: (205) 895-6791



**Charles A. Lundquist**  
Director  
**Francis C. Wessling**  
Associate Director  
**Valerie Seaquist**  
Assistant Director  
**James K. Baird**  
Principal Scientific Advisor

**AUTOMATED, MULTIPLE-SAMPLE MINILABS PROVIDE SIGNIFICANT CELL BIOLOGY AND SPACE BIOPROCESSING DATA FOR SHUTTLE MIDDECK RESEARCH.** M.L. Lewis<sup>1</sup> and J.M. Cassanto<sup>2</sup>. Consortium for Materials Development in Space, University of Alabama, Huntsville, Alabama<sup>1</sup> and Instrumentation Technology Associates, Inc., Exton, PA<sup>2</sup>

CMIX-2, the second of five Commercial MDA ITA Experiments, was launched on STS-56 on 4/8/93. The payload, returned after a nominal 9-day mission, consisted of 35 separate experiments (approximately 400 data points) in four Materials Dispersion Apparatus (MDA) Minilabs. Objectives of experiments on mouse, human, amphibian and algal cell cultures, collagen assembly, seed germination, micro-encapsulation, fluid dynamics, and protein crystal growth were to expand knowledge of low-g response and to identify potential processes/products which can benefit from space bioprocessing. One MDA was adapted for cells on coverslips, 37° incubation and triple containment. Three MDA's operated at 20° for plant and amphibian cells and other bioprocessing experiments. Significant information was obtained on mechanisms of bone and immune cell growth and function. Altered cytoskeletal morphology in both mammalian and amphibian cells confirmed cytoskeletal sensitivity to gravity. The CMIX-2 payload demonstrated low-cost basic cell biology research and potential for manufacturing in space using generic multipurpose commercially-developed hardware for space bioprocessing.

**ASGSB**



### **1993 ANNUAL MEETING ISSUE**

**CALL FOR PAPERS**

**ABSTRACT AND REGISTRATION FORMS**

**HOUSING INFORMATION**

**TENTATIVE PROGRAM**

**OCTOBER 20-23, 1993  
WASHINGTON, DC**

**1993 ASGSB MEMBERSHIP DIRECTORY**

# UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO



SANTA BARBARA • SANTA CRUZ

**MILLIE HUGHES-FULFORD, Ph.D.**

Chief, Laboratory for Cell Growth and Differentiation  
Adjunct Associate Professor of Biochemistry in Medicine

(415) 221-4810 x2749 office  
750-6940 laboratory

*Please address reply to the undersigned at:*

Laboratory for Cell Growth and Differentiation  
Mail Code 1517, Bld. 1, Rm. 110-114  
Department of Veterans Affairs Medical Center  
4150 Clement Street  
San Francisco, CA 94121

**MC3T3-E1 OSTEOBLASTS GROWN IN MICROGRAVITY ON STS-56 HAVE REDUCED CELL GROWTH, GLUCOSE UTILIZATION WITH ALTERED ACTIN CYTOSKELETON AND INCREASED PROSTAGLANDIN SYNTHESIS. M. Hughes-Fulford, K. Nelson, S. Blaug, C.G. Summer, B.D. Lukofahr, AND M.L. Lewis.** VA Medical Center and UCSF Medical Center San Francisco, CA 94121 and Consortium for Materials Development in Space and The University of Alabama in Huntsville, Huntsville, AL 35899

Spaceflight is an environmental condition where long term space inhabitants have lost up to 18% of weight bearing bone during flight. Since PGE<sub>2</sub> is postulated to be one of the key regulators of local bone formation, it is possible that a dysfunction of its synthesis or action might play a role in reduced osteoblast growth seen in spaceflight. In this study, we investigated whether bone growth was inhibited in spaceflight in the absence of hormonal changes or by changes in PGE<sub>2</sub> synthesis or its action. The osteoblasts were launched on STS-56 in a quiescent state, fetal calf sera (FCS) was added after 16 hours of microgravity exposure. After four days of growth in space we fixed the cells before return to Earth's gravity. Ground controls were treated in the same manner. Upon landing, we examined cell number, cell cytoskeleton, glucose utilization and prostaglandin synthesis. Using microscopic methods, we found that osteoblasts grew more slowly in spaceflight, with the number of cells grown in microgravity being roughly 2/3 of those on the ground. Secondly, we found the cytoskeleton of the flown osteoblasts to have altered actin structure, unlike any actin architecture we have seen before. The flown cells used less glucose than their ground controls. Finally, even with reduced glucose utilization and cell number, the flown cells had a significant 1.69 fold increase in prostaglandin synthesis when compared to ground controls. Taken together, these data suggest that microgravity directly affects the growth of osteoblasts resulting in reduced glucose utilization. The surprising increase in prostaglandin synthesis during reduced growth of the osteoblasts may be the result of an uncoupling of the prostaglandin stimulatory pathway of bone mitogenesis in microgravity.

ASGSB



## 1993 ANNUAL MEETING ISSUE

CALL FOR PAPERS

ABSTRACT AND REGISTRATION FORMS

HOUSING INFORMATION

TENTATIVE PROGRAM

OCTOBER 20-23, 1993  
WASHINGTON, DC

1993 ASGSB MEMBERSHIP DIRECTORY

**EXPOSURE TO MICROGRAVITY ALTERS PROPERTIES  
OF CULTURED MUSCLE CELLS. R. Gruener, R. Roberts &  
R. Reitstetter. U. Arizona; Tucson AZ 85724.**

Significant morphological and functional changes take place in primary cultures of *Xenopus* myocytes and neurons after rotation in the slow clinostat (i,ii). To test if these cells are sensitive to the actual microgravity of space, we flew primary cultures of myocytes in the presence of polylysine-coated polystyrene beads which, like neuron, induce acetylcholine receptor [AChR] clustering. Cell cultures were mounted in the MDA hardware (ITA technologies; John Cassanto, President), in collaboration with Dr. Marian Lewis (University of Alabama) on STS-52 and -56 flights. Cells were exposed to beads within 24 hrs of orbit entry and were fixed prior to re-entry (exposure to microgravity, before fixation, was 9d for both flights). Data analysis revealed: decreased cell and nuclear surface areas, decreased actin filament linearity, and increased actin filament "cabling". AChR aggregation was decreased in response to bead contact, and in fluorescent bungarotoxin binding area present in bead-associated clusters. Data from these flight experiments show better than 80% (by parameters assayed) concordance with results from the slow clinostat. We interpret these results to provide further evidence for 1) cellular changes in altered gravity, 2) possible involvement of the cytoskeleton in gravi-sensing, and 3) usefulness of the clinostat as an earth-bound simulation paradigm.

i. Gruener, R. & Hoeger, G. Vector-Free Gravity Disrupts Synapse Formation in Cell Culture. *Am. J. Physiol.* 258: C489-C494, 1990.

ii. Gruener, R. & Hoeger, G. Vector-Averaged Gravity Alters Myocyte and Neuron Properties. *Av. Space Env. Med.* 62:1159-1165, 1991.

Supported by a NASA contract (RG) and a Res. Associate (RaR).

ASGSB



**1993  
ANNUAL MEETING  
ISSUE**

CALL FOR PAPERS

ABSTRACT AND REGISTRATION FORMS

HOUSING INFORMATION

TENTATIVE PROGRAM

OCTOBER 20-23, 1993  
WASHINGTON, DC

## **Consortium for Materials Development in Space**

University of Alabama in Huntsville  
Huntsville, Alabama 35899  
Phone: (205) 895-6620  
Fax: (205) 895-6791

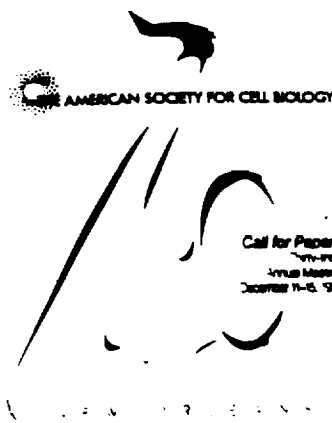


**Charles A. Lundquist**  
Director  
**Francis C. Wessling**  
Associate Director  
**Valerie Seaquist**  
Assistant Director  
**James K. Baird**  
Principal Scientific Advisor

**EFFECTS OF MICROGRAVITY ON LEUKOCYTE GROWTH CONTROL AND FUNCTION.** ((M.L. Lewis<sup>1</sup>, M.A. Principato<sup>2</sup>, B.D. Lawless<sup>3</sup>, D.R. Morrison<sup>4</sup>, W.C. Kapp<sup>5</sup>, S.L. Strobl<sup>5</sup> and A.C. Ochoa<sup>5</sup>)) Department of Biology, University of Alabama, Huntsville, AL 35899<sup>1</sup>, Immunology Branch, Food and Drug Administration, Laurel, MD 20708<sup>2</sup>, Department of Cellular Immunology, Rockefeller University, NY, NY 10021<sup>3</sup>, NASA Johnson Space Center, Houston, TX 77058<sup>4</sup> and Program Resources Inc/DynCorp, Frederick MD 21702<sup>5</sup>.

Spaceflight results in significant reduction in mitogenic activation of human T lymphocytes. Causal mechanisms are not understood though cell contact and macrophage function are implicated. We evaluated microgravity effect on lymphocyte responsiveness, cytokine secretion and signaling through the T cell receptor (TCR) complex. Human or murine T lymphocytes were exposed to: non-TCR binding Con A, an efficient activator of resting T cells in unit gravity but not in microgravity; anti-CD3 plus IL2; and superantigen Staphylococcal Enterotoxin B (SEB) in the presence of Class II presenting feeder layers in unit-gravity and on Space Shuttle flights STS-50, 52, 54, and 56. Cells were exposed to mediators for two to 94 hours followed by fixation of flown cells in microgravity. The human peripheral blood mononuclear cells (hPBMC), exposed to Con-A remained primarily in G1 during spaceflight while ground controls progressed through the replicative cycle. Glucose utilization, significantly lowered in spaceflight, confirmed reduced metabolic activity of Con-A treated cells. However, stimulation of normal hPBMC in flight with anti-CD3+IL2 resulted in activation and appreciable cytokine production (gamma-IFN, GM-CSF, IL-1beta, and IL-6) confirming previous flight results indicating significant changes in cytokine secretion. Stimulation of purified murine splenic T cells through direct engagement of the T cell receptor with the SEB superantigen or via the TCR-associated CD3 molecule using anti-CD3+IL2 was also achieved in microgravity. In both instances, the murine T cell populations transitioned into DNA synthesis (S phase) and mitotic division (G2+M), indicative of cellular proliferation. We conclude that T cell activation via the TCR-mediated pathway is unaffected by microgravity while non-TCR-mediated activation, confirmed by poor proliferative response to Con A, does not promote cell division in microgravity. Our results suggest that microgravity may permit separation of proliferative signaling and T cell responsiveness and thus provide an unparalleled opportunity to investigate basic cellular mechanisms controlling growth and function.

Submitted to the 1993 Annual Meeting of the American Society for Cell Biology



**PRE-METATARSAL ORGAN CULTURE IN MICROGRAVITY. Brenda J. Klement and Brian S. Spooner, Kansas State University.**

A potentially debilitating effect of spaceflight is the demineralization and abnormal deposition of bone ECM. Most of the data on mineralizing tissue in microgravity are from adult bone. Less is known about growing bones with active growth plates, and even less is known about microgravity effects on formation and initial calcification of the growth plate. We have flown an explant organ culture system, using the pre-metatarsals from embryonic mice, in experiments on two shuttle flights. In ground based culture, pre-metatarsal mesenchyme is removed from day 13 embryos. By culture day 3, the cells have differentiated into chondrocytes and organized into cartilage rods. With further culture, some cells terminally differentiate into hypertrophied chondrocytes. The matrix surrounding the hypertrophied cells calcifies, documented in our cultures by alizarin red staining, establishing the initial site for osteoblasts to begin bone deposition. For microgravity experimentation, we modified the culture system for use in available flight hardware. On STS-54, in the CGBA payload, we did our culture experiments in Bioserve FPAs. Pre-metatarsals were placed on the membranes of Millicell units, covered with Matrigel, and cultured in medium-filled FPA chambers. At 8 hours MET, some cultures were fixed and some were given additional medium. At MET 5 days 2 hours, all cultures were fixed. Histology shows differentiation during flight into cartilaginous rods. No conspicuous differences were noted between flight tissues and ground controls. A second flight opportunity was on STS-56, where we flew older pre-metatarsals in the CMIX payload. Little evident growth occurred in flight or in ground controls, but the tissue remained healthy for the 4 day experiment.



**Center for Basic Cancer Research**

Division of Biology  
Ackert Hall  
Manhattan, Kansas 66506-4903  
913-532-6705  
FAX: 913-532-6653

**ASGSB**



**1993  
ANNUAL MEETING  
ISSUE**

**CALL FOR PAPERS**

**ABSTRACT AND REGISTRATION FORMS**

**HOUSING INFORMATION**

**TENTATIVE PROGRAM**

**OCTOBER 20-23, 1993  
WASHINGTON, DC**

**1993 ASGSB MEMBERSHIP DIRECTORY**

**Human Splenic B Cell Activation *in vitro* is Not Impaired by Microgravity ( $\mu$ g).** G.A. Neil<sup>1</sup>, L.A. Love-Homan<sup>1</sup>, C. Summer<sup>2</sup>, D.W. Sammons<sup>3</sup> and M.L. Lewis<sup>2</sup>. Universities of Iowa, Alabama, Huntsville<sup>2</sup>, and Arizona<sup>3</sup>.

We tested the hypothesis that human splenic B lymphocytes are resistant to *in vitro* activation in microgravity ( $\mu$ g) by stimulation and fixation aboard STS-56.

**Methods:** Normal human splenocytes derived from living organ donors were prepared by density gradient centrifugation and kept frozen until use. The cells were thawed and loaded into the wells of a Materials Dispersion Apparatus (MDA, ITA) at L-30 hr and maintained at 20°. The cells were activated in flight at 37° with one of: medium, lipopolysaccharide (LPS), *S. aureus* cowan (SAC),  $\alpha$ -CD3 or CD40 ligand at L+16 hr then fixed at L+5 days in modified PLP. Simultaneous ground-based controls were performed. Staining and cell cycle analysis were performed post-flight using propidium iodide staining and FACS.

**Data:** Substantial activation of human B cells was evident with soluble (LPS), membrane bound (SAC, CD40 ligand) and cell-contact ( $\alpha$ -CD3) reagents. The magnetic beads used for mixing the cultures exhibited the ability to activate the cultures, increasing "background" activation. As shown below, no significant difference in the fraction of cells entering cell cycle in flight ( $\mu$ g) vs. ground-based controls was evident.

Activating Agent	% Cells in Cycle:	
	Ground-Base	Flight
Medium	46.5	51.4
LPS	81.8	75.4
SAC	84.6	81.4
$\alpha$ -CD3	94.4	99.8
CD40 Ligand	88.8	88.8

**Conclusion:** Under these experimental conditions, *in vitro* activation of human splenic B cells was not impaired by  $\mu$ g.

Supported by NASA 18433

ASGSB

1993  
ANNUAL MEETING  
ISSUE

CALL FOR PAPERS

ABSTRACT AND REGISTRATION FORMS

HOUSING INFORMATION

TEMPERATURE PROGRAM

OCTOBER 20-23, 1993  
WASHINGTON, DC

1993 ASGSB MEMBERSHIP DIRECTORY

Garry Neil, M.D.  
Oakdale Research Park  
Room B164  
2501 Crosspark Road  
Coralville, IA 52241

**Superantigen-mediated T cell Receptor  
Stimulation in Zero Gravity.**

M.A.Principato\*, B.D. Lawless^, M. Lewis o.  
\*Immunobiology Branch, CFSAN, Food and Drug  
Administration, Laurel, MD. ^Rockefeller  
University, New York. o Univ. of Alabama,  
Huntsville, AL.

We examined the responses of purified murine T cells following potent *in vitro* stimulation of the T cell receptor complex (TcR) during the STS-56 mission of April 1993. Murine splenic T cells were stimulated by engagement of the TcR associated-CD3 molecule using an anti-CD3 monoclonal or by direct stimulation of VB8 and VB6 T cell subpopulations with a superantigen, Staphylococcal Enterotoxin (SEB). Proliferative responses were assayed by measurement of accumulated DNA content via flow cytometry as an indicator of progression through the mitotic cell cycle.

The results demonstrate that direct stimulation of the CD3-TcR complex by the 2C11 monoclonal or engagement of the appropriate VB families by SEB resulted in proliferation in matched ground controls and cultures activated in orbit. These data indicate that zero gravity does not preclude primary activation of T lymphocytes via direct engagement of the TcR complex, and that the stoichiometry between SEB, class II molecules, and the appropriate T cell receptor is maintained in zero gravity.

Mary Ann Principato, Ph.D.  
Senior Investigator  
Immunobiology Branch  
Food and Drug Administration  
8301 Muirkirk Road  
Laurel, MD 20708

ASGSB

1993 ANNUAL MEETING ISSUE
CALL FOR PAPERS
ABSTRACT AND REGISTRATION FORMS
HOUSING INFORMATION
TENTATIVE PROGRAM
OCTOBER 20-23, 1993 WASHINGTON, DC

1993 ASGSB MEMBERSHIP DIRECTORY